VOL. XL. SEPTEMBER, 1908. NO. 3.

AMERICAN CHEMICAL JOURNAL

TRIPHENYLINDENE AND SOME OF ITS DERIVATIVES.

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In an earlier paper[1] I described the preparation of diphenylbenzalacetophenone by boiling α-bromtriphenylpropiophenone with a strong alcoholic solution of potassium hydroxide:

$$(C_6H_5)_2CHCBr(C_6H_5)COC_6H_5 + KOH = (C_6H_5)_2C:C(C_6H_5)COC_6H_5 + KBr + H_2O.$$

This procedure invariably gives, in addition to the unsaturated ketone, a small quantity of a substance that separates from solution in large colorless crystals and has a melting point that is considerably higher than that of the ketone. The filtrate from a number of preparations of unsaturated ketone yielded a sufficient quantity of this substance for an analysis and a determination of its molecular weight. The results are expressed by the formula $C_{29}H_{24}O$.

A compound having the composition $C_{29}H_{24}O$ might be formed by addition of alcohol to the unsaturated ketone and loss of water from the product, but an experiment showed that the ketone is not affected by prolonged boiling with alcoholic

[1] THIS JOURNAL, 38, 559.

potassium hydroxide. The substance must, therefore, be formed from the bromoketone—either by replacement of bromine with ethoxyl and subsequent elimination of water or, *vice versa*, by loss of water succeeded by replacement of bromine.

By keeping an alcoholic solution of α-bromtriphenylpropiophenone at a low temperature for a long time it is possible to replace bromine with ethoxyl. Not a trace of the compound $C_{29}H_{24}O$ is formed under these conditions and I have found no way of getting this substance from the ethoxyketone. It is, however, easy to eliminate water from the bromoketone. When it is heated either by itself or in solution in indifferent solvents it loses one molecule of water and passes almost quantitatively into the bromide $C_{27}H_{19}Br$. When the bromide is dissolved in alcohol it gives the compound $C_{29}H_{24}O$. This is, therefore, an ether formed from α-bromtriphenylpropiophenone in accordance with the equations

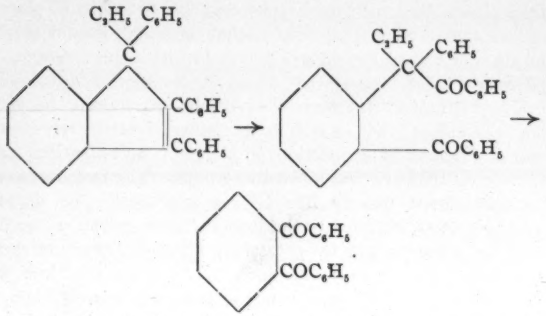
$$\text{I. } (C_6H_5)_2CHCBr(C_6H_5)COC_6H_5 - H_2O = C_{27}H_{19}Br;$$
$$\text{II. } C_{27}H_{19}Br + C_2H_5OH = C_{27}H_{19}OC_2H_5 + HBr.$$

The structure of the ether was established by the following transformations: Both the bromide and the ether are readily oxidized with chromic acid dissolved in acetic acid. The principal oxidation products are in each case benzoic acid and *o*-dibenzoylbenzene. The formation of the latter in this reaction indicates the presence of a condensed ring system containing one benzene ring and shows the position of two of the phenyl groups. In order to establish the character of the second ring and to locate the remaining groups it was necessary to introduce a hydrocarbon residue in place of bromine or ethoxyl. This was accomplished with ethylmagnesium bromide which reacts with the bromide in accordance with the equation

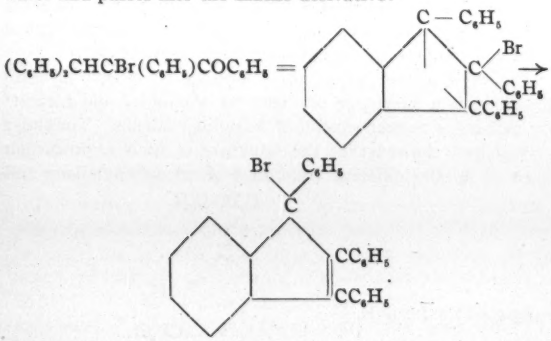
$$C_{27}H_{19}Br + C_2H_5MgBr = C_{27}H_{19}C_2H_5 + MgBr_2.$$

The hydrocarbon resulting from this reaction gives only one product when oxidized with chromic acid at the ordinary temperature—a diketone with all the hydrocarbon residues intact. On further oxidation in boiling acetic acid, the dike-

tone gives *o*-dibenzoylbenzene. These results show that the hydrocarbon is a derivative of 1,2,3-triphenylindene:



The bromide $C_{27}H_{20}Br$ is therefore 1-brom-1,2,3-triphenylindene and it is evident that there must be a shifting of the bromine atom when α-bromtriphenylpropiophenone loses water and passes into the indene derivative:

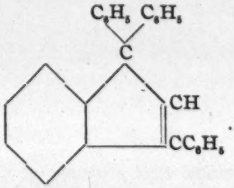


It is remarkable that a change so complicated as this one should take place far below the melting point of the bromoketone, and without more than a trace of by-products. I have made a number of experiments for the purpose of determining

to what extent this reaction can be used for making other indene derivatives and have found that the reaction does not take place either with the ketone that has hydrogen in place of bromine, or with bromoketones that have hydrogen or alkyl in place of any of the phenyl groups in bromtriphenylpropiophenone.

By starting with bromtriphenylindene it is easy to get triphenylindene itself as well as a number of derivatives that are of interest in connection with recent investigations of substances that are rich in phenyl groups. The hydrocarbon behaves almost exactly like triphenylmethane. The single hydrogen atom attached to the indene ring is readily replaced both by halogens and by potassium. The hydrocarbon can be oxidized directly to the carbinol. The halogen substitution products are slightly less reactive than the corresponding triphenylmethane derivatives but give the characteristic reactions of those substances. Thus the yellow bromide forms double salts with metallic halides, reacts with alcohols to form ethers, and gives, with metals, an unsaturated compound analogous to "triphenylmethyl." Similarly, the carbinol obtained by replacing bromine with hydroxyl gives the typical color and condensation reactions of triphenylcarbinol.

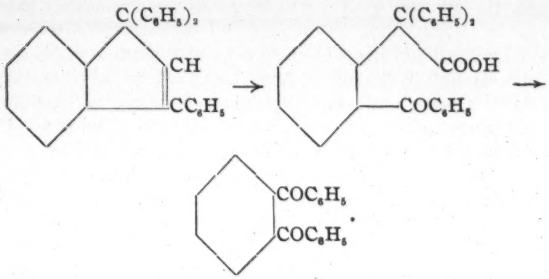
The only phenyl substitution product of indene hitherto described is a substance obtained by Vorländer and Siebert[1] by molecular rearrangement of tetraphenylallene. Vorländer and Siebert showed that the structure of their hydrocarbon must be totally different from that of tetraphenylallene and suggested the formula



As the substance has the same melting point as the triphenylindene described in this paper and as the oxidation products

[1] Ber. d. chem. Ges., **39**, 1030.

obtained by heating the two hydrocarbons with chromic acid in acetic acid also melt at the same temperature, it was necessary to repeat the experiments with tetraphenylallene. The results show that the substances are different and that the formula proposed by Vorländer and Siebert is correct. By cautious oxidation it is possible to convert their hydrocarbon into a ketonic acid that readily loses carbon dioxide and that can be oxidized to *o*-dibenzoylbenzene:



The behavior of this hydrocarbon is quite different from that of 1,2,3-triphenylindene; it cannot be oxidized to the carbinol, it has no hydrogen that is replaceable by metals, the bromine substitution product is colorless, and the bromine is not available for metathetical reactions.

EXPERIMENTAL PART.

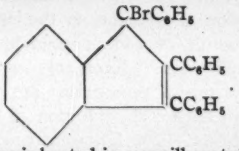
Preparation of Bromtriphenylpropiophenone.—As triphenylpropiophenone does not react with bromine[1] it is necessary to make the bromine substitution product indirectly, by the action of bromine on the magnesium derivative obtained as a result of adding phenylmagnesium bromide to benzaldeoxybenzoin.[2] Even this method gives very poor yields unless special precautions are taken to avoid rise in temperature during the addition of bromine and while isolating the product. The following procedure gives satisfactory results:

[1] THIS JOURNAL, **34**, 572.
[2] *Ibid.*, **36**, 195.

One hundred grams of finely powdered benzaldeoxybenzoin is added in small quantities to an ethereal solution of phenylmagnesium bromide made from 20 grams of magnesium. The solution is boiled for a few minutes, then cooled with an efficient freezing mixture, and treated with 120 grams of bromine that has been dried by distillation from phosphorus pentoxide. The bromine should be added as slowly as possible from a dropping funnel that delivers very small drops and the mixture must be shaken constantly to avoid local rise in temperature. As soon as all the bromine has been added, the liquid is poured into a freezing mixture of ice and concentrated hydrochloric acid, contained in a separating funnel, shaken until the solid product begins to separate, then diluted with 2 to 3 times its volume of low-boiling ligroin that has been cooled in a freezing mixture.

By constant and vigorous shaking for an hour it is possible to get almost the entire product in the form of a fine crystalline deposit. After filtration this is washed—first with water, then with a mixture of ether and ligroin (1 to 4), and finally with low-boiling ligroin. The colorless powder left after drying over sulphuric acid in a vacuum contains small quantities of triphenylpropiophenone and tetraphenylpropenone. These substances may be removed by recrystallization from a mixture of chloroform and ligroin but for most purposes the carefully washed and dried product is sufficiently pure. The yield is satisfactory—120 to 140 grams from 100 grams of benzaldeoxybenzoin. By evaporating the filtrate and boiling the oily residue with alcoholic potassium hydroxide the bromoketone left in solution can be transformed into the sparingly soluble triphenylpropenone.

1-Brom-1,2,3-triphenylindene,



When bromtriphenylpropiophenone is heated in a capillary tube it melts at 124° and immediately decomposes with evolution

of gas, leaving an oily residue that is orange when hot, yellow when cold. The same decomposition takes place slowly when a clear, colorless crystal is heated on a steam bath. The crystal first turns yellow, then melts to a pale yellow liquid that gradually becomes darker in color and finally solidifies after heating for 50 to 60 hours. At the ordinary temperature the solid ketone seems to be stable but its solutions in indifferent solvents slowly turn yellow. A saturated solution of 10 grams in absolute ether, to which a small piece of fused calcium chloride had been added for the purpose of removing any water liberated, deposited 0.75 gram of bromide in 7 months. The same product was obtained in all these experiments—a yellow solid melting at 129°. To prepare this substance in quantity 50 gram portions of the bromoketone were heated for a short time to 150°–160° in wide beakers. The orange colored oil was poured, while hot, into a small quantity of boiling chloroform, the solution diluted with 0.5 volume of acetone and 4 to 5 volumes of ligroin (40°), and cooled in a freezing mixture. The product separated in minute yellow plates melting at 127°. To get a perfectly pure product it is best to recrystallize the substance from alcohol-free ether, from which it separates in large transparent plates melting at 129°.

Analysis:

I. 0.1198 grams ubstance gave 0.3373 gram CO_2 and 0.0485 gram H_2O.

II. 0.1319 gram substance gave 0.3700 gram CO_2 and 0.0534 gram H_2O.

	Calculated for $C_{21}H_{19}Br$.	Found. I.	Found. II.
C	76.6	76.9	76.5
H	4.5	4.5	4.5

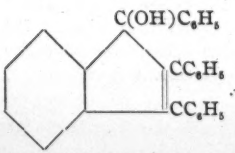
Bromtriphenylindene is stable in the air. It is readily soluble in chloroform and carbon tetrachloride, moderately in acetone and ethyl acetate, sparingly in ether and ligroin. It reacts rapidly with alcohol and it is slowly reduced by acetone. Pure cold sulphuric acid dissolves it only slightly while the hot acid decomposes it, forming hydrobromic acid

and an intensely red solution that gives the carbinol when diluted with water. The solution in liquid sulphur dioxide has the same color as the solid but readily gives metathetical reactions with salts. Thus dry silver sulphate reacts instantaneously, forming the calculated amount of silver bromide and a brilliant red sulphate. By using a very dilute solution it was possible to get the sulphate free from bromide, but the substance is so extremely sensitive to moisture that I failed to get consistent analytical results.

Bromtriphenylindene, like triphenylmethyl chloride, forms double salts with metallic halides. The dark red crystalline compound with aluminium bromide obtained by bringing the components together in nitrobenzene was analyzed.

I. 0.2011 gram substance gave 0.2191 gram AgBr.
II. 0.2504 gram substance gave 0.0188 gram Al_2O_3.

	Calculated for $C_{27}H_{19}Br.AlBr_3$.	Found. I.	II.
Br	46.4	46.3	..
Al	3.8	....	4

Oxytriphenylindene, 

.—Bromtriphenylindene can be boiled with water and even with strong aqueous potassium hydroxide without undergoing appreciable change, but water or sodium acetate rapidly decolorizes the yellow solution in hot acetic acid. In each case bromine is replaced with hydroxyl. When the colorless solution is poured on ice the carbinol separates as a paste, which slowly passes into an amorphous solid. This can be filtered and washed but it is extremely difficult to get it in crystalline form as all the common solvents deposit it as an oil. The best results were obtained by dissolving it in glacial acetic acid, diluting the solution with water until a slight cloudiness appeared, redissolving this precipitate with a few drops of acetic acid,

and allowing the solution to evaporate spontaneously. In the course of a few weeks most of the substance separated in lustrous prisms that melt sharply at 129°.

Analysis:

0.1110 gram substance gave 0.3670 gram CO_2 and 0.0522 gram H_2O.

	Calculated for $C_{27}H_{20}O$.	Found.
C	90.0	90.2
H	5.5	5.3

Oxytriphenylindene is readily soluble in all common solvents except water and low-boiling ligroin. It also dissolves readily in cold concentrated sulphuric acid. The solution has an intensely red color which persists on heating. The carbinol does not react with boiling acetyl chloride and no acetyl derivative was obtained by prolonged boiling with acetic anhydride and sodium acetate. The presence of the hydroxyl group is shown by the behavior towards ethylmagnesium bromide. It liberates an equivalent quantity of ethane and is recovered unchanged when the resulting magnesium derivative is decomposed with water.

The *ethers* of oxytriphenylindene are very easily obtained by adding a few drops of concentrated sulphuric acid to its solutions in alcohols and boiling for a short time or by simply dissolving bromtriphenylindene in the boiling alcohols. As they are sparingly soluble in alcohol and crystallize well they served as a convenient means of detecting the carbinol in reaction products.

The *methyl ether*, *methoxytriphenylindene*, $C_{28}H_{22}O$, crystallizes in tables melting at 153°. Its solubility in various solvents and its behavior towards reagents are similar to those of the ethyl ether described below.

Analysis:

0.1422 gram substance gave 0.4667 gram CO_2 and 0.0788 gram H_2O.

	Calculated for $C_{28}H_{22}O$.	Found.
C	89.8	89.5
H	5.9	6.1

The *ethyl ether*, $C_{20}H_{24}O$, is readily soluble in chloroform and ethyl acetate, moderately in acetone and boiling ether, sparingly in alcohol and ligroin. It separates from acetone in large lustrous prisms melting at 172°.

Analysis:

I. 0.1100 grams ubstance gave 0.3630 gram CO_2 and 0.0627 gram H_2O.

II. 0.1278 gram substance gave 0.4202 gram CO_2 and 0.0725 gram H_2O.

	Calculated for $C_{20}H_{24}O$.	Found. I.	II.
C	89.7	90.0	89.6
H	6.2	6.4	6.3

The molecular weight was determined in boiling acetone. K = 1710.

Solvent. Grams.	Substance. Grams.	Elevation of boiling point.	Molecular weight.
36.40	0.6210	0.078	370
36.40	1.2550	0.160	366
36.40	1.9115	0.238	378

Calculated for $C_{20}H_{24}O$, 388

The ether does not reduce potassium permanganate dissolved in acetone but it is readily oxidized with chromic acid dissolved in hot glacial acetic acid. Ten grams of the substance were dissolved in 100 grams of boiling acetic acid and treated with small quantities of chromic acid until a permanent color of the acid remained in the liquid. The solution was then poured on ice and allowed to stand until the precipitated paste solidified. The solid was washed with water, dried, and crystallized from a mixture of acetone and alcohol. It separated in colorless tables melting at 148°.

Analysis:

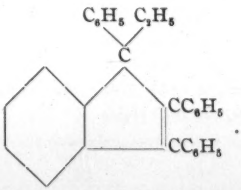
I. 0.1189 gram substance gave 0.3649 gram CO_2 and 0.0525 gram H_2O.

II. 0.1321 gram substance gave 0.4055 gram CO_2 and 0.0584 gram H_2O.

	Calculated for $C_{20}H_{14}O_2$.	Found. I.	II.
C	83.9	83.7	83.7
H	4.9	4.9	5.0

The oxidation product has the composition of *o*-dibenzoylbenzene, but this, according to Zincke, is pale yellow in color and melts at 146°. For the purpose of comparison I made *o*-dibenzoylbenzene by oxidizing *o*-dibenzylbenzene with chromic acid. The difficulties experienced by Zincke and by Wehnen in preparing this ketone can be obviated by using an aqueous solution of chromic acid. Ten grams of dibenzylbenzene were dissolved in 100 cc. of boiling glacial acetic acid contained in a flask connected with an inverted condenser. A solution of 10 grams of chromic acid in the minimum quantity of cold water was added drop by drop from a funnel connected with the top of the condenser. The mixture was boiled until the color of chromic acid had disappeared, then poured into water, and the products separated in the usual way. The yield was 8 grams. By recrystallization from acetone and alcohol it was obtained in colorless tables that melted at 148°. A mixture of *o*-dibenzoylbenzene and the oxidation product obtained from ethoxytriphenylindene melted at the same temperature.

1-Ethyl-1,2,3-triphenylindene,



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This substance was made in the hope of getting an oxidation product intermediate between the indene derivative and *o*-dibenzoylbenzene. It was easily obtained by adding bromtriphenylindene to excess of ethereal ethylmagnesium bromide and boiling until the resulting solution was colorless. The product was isolated in the usual way and purified by recrystallization from acetone and alcohol. It was thus obtained in plates melting at 108°.

Analysis:

0.1125 gram substance gave 0.3860 gram CO_2 and 0.5645 gram H_2O.

	Calculated for $C_{29}H_{24}$.	Found.
C	93.55	93.6
H	6.45	6.6

The hydrocarbon is readily soluble in chloroform and acetone, moderately in alcohol and ether, sparingly in ligroin. It separates very slowly from solution but forms large and well-defined crystals. The yield was 13 grams from 15 grams of the bromide.

Benzoyltriphenylbutyrophenone, $C_6H_5COC_6H_4C(C_2H_5)(C_6H_5)COC_6H_5$.—Ethyltriphenylindene does not reduce potassium permanganate dissolved in acetone. It was oxidized by adding chromic acid in small quantities to a cold solution in acetic acid as long as the color rapidly changed to green after each addition. The solution was then poured into ice water which precipitated a paste that remained liquid for a long time but solidified when rubbed in contact with ether. The solid was purified by crystallization from acetone. Yield of pure product, 10.2 grams.

Analysis:

I. 0.1190 gram substance gave 0.3732 gram CO_2 and 0.0635 gram H_2O.

II. 0.1314 gram substance gave 0.4147 gram CO_2 and 0.0706 gram H_2O.

	Calculated for $C_{29}H_{24}O_2$.	Found. I.	Found. II.
C	86.1	85.5	86.0
H	5.9	5.9	5.9

Benzoyldiphenylbutyrophenone crystallizes in large shining tables melting at 180°. It is readily soluble in chloroform and acetone, sparingly in alcohol and ether. When boiled with excess of chromic acid in acetic acid it is slowly oxidized to *o*-dibenzoylbenzene and *o*-benzoylbenzoic acid.

Triphenylindenyl Peroxide, $C_{27}H_{19}O—OC_{27}H_{19}$.—It seemed probable, in view of the close resemblance between the substances described above and the corresponding derivatives of triphenylmethane, that it would be possible to eliminate bromine from the indene derivative and get a compound

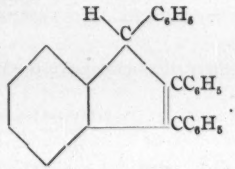
analogous to "triphenylmethyl." This is the case. When the yellow solution of the bromide in dry benzene is shaken with mercury or, better, with amalgamated zinc, it rapidly changes in color from yellow to orange or red and, in case zinc amalgam is used, a thin film of a bright red double salt with zinc bromide forms on the metal. The double salt disappears after a time but the color of the solution is permanent. When the colored solution is allowed to evaporate in contact with dry air the color soon fades and a colorless crystalline deposit forms on the sides of the vessel. This is the peroxide. It was purified by recrystallization from a mixture of chloroform and ether.

Analysis:

0.1310 gram substance gave 0.4337 gram CO_2 and 0.0650 gram H_2O.

	Calculated for $(C_{27}H_{19}O)_2$.	Found.
C	90.25	90.3
H	5.57	5.4

The peroxide was obtained in small shining prisms that decompose at about 155°. It is readily soluble in chloroform, sparingly in acetone, alcohol, and ether. Its solution in sulphuric acid has the same color as that of the carbinol in the same solvent. From this solution water precipitates some carbinol while alcohol gives almost the calculated quantity of ethoxytriphenylindene.

1,2,3-Triphenylindene, 

. — The results of the preceding experiment show that metals eliminate halogen from the bromide and form an unsaturated compound. This was utilized for passing from the halogen derivative to the hydrocarbon. In the presence of a small quantity of iodine magnesium reacts very readily with an ethereal solution of

the bromide but, apparently, without forming a trace of magnesium derivative. The experimental conditions were varied in every manner suggested by the results obtained by Schmidlin and by Tschitschibabin with triphenylchlormethane, but no magnesium derivative could be detected in the solution.

When the unsaturated compound, contained in the dark red solution obtained with magnesium, is oxidized with air or oxygen it gives almost the calculated amount of peroxide. When it is treated with water it gives approximately equal weights of triphenylindene and oxytriphenylindene. And when it is reduced it gives almost exclusively triphenylindene. The reduction was carried out as follows: Ten grams of the finely powdered halogen compound were suspended in 75 cc. of anhydrous ether contained in a flask that was connected with an inverted condenser and with a hydrogen generator. The ether was boiled for a short time in order to saturate the solution, the air in the apparatus replaced with dry hydrogen, and 5 grams of magnesium and a crystal of iodine added through the condenser. The reaction began at once and proceeded quietly without further heating. When all of the halogen compound had disappeared the liquid was cooled and cautiously treated with enough concentrated hydrochloric acid to dissolve the excess of magnesium. The ethereal layer was washed, dried over fused sodium sulphate, and the ether allowed to evaporate. It deposited 6 grams of solid and a small quantity of oil. The solid was easily purified by crystallization from acetone.

Analysis:

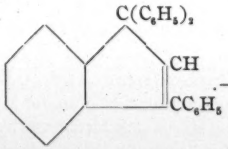
0.1277 gram substance gave 0.4370 gram CO_2 and 0.0678 gram H_2O.

	Calculated for $C_{21}H_{20}$.	Found.
C	94.2	93.9
H	5.8	5.8

Triphenylindene separates from ether or acetone in large colorless prisms melting at 135°. It is readily soluble in chloroform and ether, moderately in acetone and alcohol, sparingly in ligroin. It is very slightly soluble in cold sul-

phuric acid and its solution in the warm acid is colorless. The hydrocarbon does not react with bromine dissolved in chloroform or boiling carbon tetrachloride but substitution takes place when bromine vapor is passed through the substance heated to 150°–160°. The only product is the yellow bromide described above.

Triphenylindene is readily oxidized with chromic acid. At the ordinary temperature the principal product is the carbinol. Thus 5 grams of chromic acid were added to 5 grams of the hydrocarbon dissolved in 100 grams of glacial acetic acid and the mixture allowed to react for 24 hours. The solution was then diluted with much water and extracted with ether. After washing with sodium carbonate and drying with sodium sulphate the ether was allowed to evaporate. It deposited a small quantity of *o*-dibenzoylbenzene and an oil that gave 4.8 grams of ethoxytriphenylindene when boiled with alcohol and a few drops of concentrated sulphuric acid. This shows that 4.5 grams of carbinol were formed by direct oxidation of the hydrocarbon. *o*-Dibenzoylbenzene is the only product that is obtained when the oxidation is carried out in boiling acetic acid.

1,1,3-Triphenylindene, 

.—The tetraphenylallene used in the preparation of this hydrocarbon was made from tetraphenylpropyl alcohol by the excellent method of Vorländer and Siebert.[1] It was transformed into the indene derivative by passing dry gaseous hydrogen chloride into its solution in boiling glacial acetic acid. The first few bubbles of gas developed a red color in the solution. This deepened as the concentration of the acid increased, then began to fade, and in a remarkably short time—less than 5 minutes for 50 grams of substance—the solution became colorless and the transformation was complete. The solution was poured into

[1] Ber. d. chem. Ges., **39**, 1030.

ice water, the solid product removed, washed with water, and recrystallized from methyl alcohol. It melted at 135°, the same temperature at which 1,2,3-triphenylindene melts, but a mixture of the two began to melt at about 104°.

Vorländer and Siebert found that chromic acid in boiling acetic acid oxidizes the hydrocarbon and forms a product melting at 146°–148°. This is *o*-dibenzoylbenzene.

0.1309 gram substance gave 0.4078 gram CO_2 and 0.0580 gram H_2O.

	Calculated for $C_{20}H_{14}O_2$.	Found.
C	83.9	83.8
H	4.9	5.0

These results differ somewhat from those obtained by Vorländer and Siebert but a comparison of the oxidation product with some *o*-dibenzoylbenzene made from dibenzoylbenzene established the identity of the substances.

As the hydrocarbon gives *o*-dibenzoylbenzene it must be an indene derivative with phenyl groups in the 1 and 3 positions. By oxidizing at a lower temperature it is possible to get an intermediate oxidation product, the formation of which proves the presence of two phenyl groups in the 1 position.

o-Benzoyltriphenylacetic Acid, $C_6H_5COC_6H_4C(C_6H_5)_2CO_2H$.—A solution of 12 grams of chromic acid in the minimum quantity of cold water was added drop by drop to a solution of 15 grams of the hydrocarbon in glacial acetic acid. The mixture was kept cool with running water for 18 hours, then poured into ice water. The pasty precipitate was washed with water, then dissolved in ether and shaken with a saturated solution of sodium carbonate. This precipitated a sparingly soluble sodium salt that separated in needles. The salt is almost insoluble in cold water and is decomposed by boiling water. It was washed with water and recrystallized by solution in methyl alcohol and cautious addition of ether. It was thus obtained in small colorless prisms, melting with decomposition at about 130°.

Analysis:

0.3220 gram substance gave 0.0542 gram Na_2SO_4.

	Calculated for $C_{27}H_{19}O_3Na.H_2O$.	Found.
Na	5.32	5.5

In order to get the acid the sodium salt was suspended in ether and shaken with dilute hydrochloric acid. The ethereal layer was dried and the ether allowed to evaporate. The resulting solid was purified by crystallization from a mixture of ether and ligroin. It separated in small prisms that melted with decomposition at 230°.

Analysis:

0.1311 gram substance gave 0.3971 gram CO_2 and 0.0614 gram H_2O.

	Calculated for $C_{27}H_{20}O_3$.	Found.
C	82.7	82.6
H	5.1	5.2

The formation of *o*-benzoyltriphenylacetic acid and *o*-dibenzoylbenzene, by oxidation, shows that the formula proposed by Vorländer and Siebert is correct and that tetraphenylallene very readily undergoes rearrangement to 1,1,3-triphenylindene.

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[Contributions from the Sheffield Laboratory of Yale University.]

CLVIII. — RESEARCHES ON PYRIMIDINES: SYNTHESIS OF CYTOSINE-5-CARBOXAMIDE.

[THIRTY-SIXTH PAPER.[1]]

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In our previous papers on the synthesis of the 5-carboxyl derivatives of uracil[2] and cytosine[3] we have shown that Claisen's ethoxymethylenemalonic ester,[4] $C_2H_5OCH = C(CO_2C_2H_5)_2$,

[1] A complete list of our previous papers on pyrimidines up to the end of the year 1907 is given at the end of this article.

[2] THIS JOURNAL, **37**, 392 (1907).

[3] *Ibid.*, **38**, 594 (1907).

[4] Ann. Chem. (Liebig), **297**, 75 (1897).

condenses in alkaline solution with 2-ethylpseudothiourea, $H_2N—C(SC_2H_5) = NH$, giving 2-ethylmercapto-5-carbethoxy-6-oxypyrimidine (Formula I., on page 237). The condensation is especially smooth. We have succeeded in greatly increasing the yield of pyrimidine over the amount obtained in our first experiments and this condensation now offers an excellent starting point for further syntheses. We have also previously shown that 2-ethylmercapto-5-carbethoxy-6-oxypyrimidine is easily saponified by alkali and the yield of 2-ethylmercapto-6-oxypyrimidine-5-carboxylic acid, II., is very good.

We now find that with proper precautions this mercapto acid can be practically quantitatively converted into the acid chloride of 2-ethylmercapto-6-chlorpyrimidine-5-carboxylic acid, III. This acid chloride is a very reactive substance and, as might be expected, the chlorine attached to the CO group is more reactive than that in the 6 position.

When the dichloride is treated with cold aqueous ammonia one chlorine atom is replaced by the amino group and 2-ethylmercapto-6-chlorpyrimidine-5-carboxamide, IV., results. This is proved by the fact that on treating the chloramide with sodium ethylate an ethoxy derivative, V., is obtained which is isomeric and not identical with 2-ethylmercapto-5-carbethoxy-6-aminopyrimidine, X.

When the dichlorpyrimidine is warmed with concentrated aqueous ammonia on the steam bath, both chlorine atoms are removed and it is quantitatively converted into 2-ethylmercapto-6-aminopyrimidine-5-carboxamide, VI.

We have found that this acid amide combines with bromine with the evolution of heat and that the product has the properties and composition of a dibrom addition product, VII. When it was treated with alkali it regenerated unaltered 2-ethylmercapto-6-aminopyrimidine-5-carboxamide.

When it was heated under certain conditions, its loss in weight and percentage of nitrogen corresponded with that required for the formation of a monobromamide. If a monobromamide was formed here it might be expected that, in common with such substances, on treatment with alkali the bromamide group, RCONHBr, would undergo a rearrange-

ment into the carbonamide form, RNCO. In this case the reactive grouping would probably unite with the adjacent amino group in the 6 position, forming a five membered ring, with the result that an 8-oxypurine would be obtained.

When the product or mixture obtained by heating the dibrom addition product was treated with aqueous alkali, cytosine-5-carboxylic acid, VIII., sometimes 2-ethylmercapto-6-aminopyrimidine-5-carboxamide, VI., and a product free from sulphur was obtained. This latter substance and its salts gave results on analysis which closely agreed with the calculated for 2,8-dioxypurine, XI., the only one of the dioxypurines that has not been described. The empirical formula of 2,8-dioxypurine, $C_5H_4O_2N_4$, differs from cytosine-5-carboxamide, IX., $C_5H_6O_2N_4$, by only two hydrogen atoms. The percentage composition of these substances is therefore so closely similar that the analysis did not decide which one of the two we were dealing with. In our previous work we had reason to believe that we had obtained cytosine-5-carboxamide, IX. With the object of preparing this substance, the behavior of the ethyl ester of cytosine-5-carboxylic acid, or 5-carbethoxycytosine, XII., with aqueous ammonia was examined at that time and it was found that this ether showed an exceptional inertness. On heating at 140° to 150° for two hours, about 25 per cent remained unaltered. On concentrating the mother liquor more soluble material crystallizing in needles was obtained. The analytical results in this case and the method of preparation were then taken to indicate that the expected cytosine-5-carboxamide had been obtained. Owing to the poor yield, nothing further was done with this substance.

This material was not identical with that obtained in the present work. The facts then seemed to show that the material obtained from the brom addition product, VII., was 2,8-dioxypurine. The question was finally settled in the negative, as follows: The material obtained by heating the dibromamide and then treating with alkali was carefully freed from 2-ethylmercapto-6-aminopyrimidine-5-carboxamide and cytosine-5-carboxylic acid. It was then boiled with strong hydrochloric acid and found to completely decompose into cytosine-5-carboxylic acid.

This shows that no Hofmann rearrangement had taken place and that the present substance is the true cytosine-5-carboxamide.

The inertness of 5-carbethoxycytosine, and also of 2,6-diamino-5-carbethoxypyrimidine,[1] on heating with ammonia, is comparable with that of the dialkylmalonic esters.[2] The ammonium salts of these acids break down with the evolution of carbon dioxide, and since 5-carbethoxycytosine is a substituted malonic acid derivative this offers an explanation of our previous results. The material formerly viewed as cytosine-5-carboxamide was probably impure or partly hydrous cytosine.

It is probable that no monobromamide was formed on heating the dibrom addition product and that the dibrom addition product has the bromine atoms attached to the double bond between the 4 and 5 positions. Similar additions of bromine to the double bond have been observed in other cases in this laboratory, *e. g.*, thymine and 4,5-dimethyluracil. The compounds in general have little stability. An unusually stable dibrom addition product of this sort, prepared from 2-phenylhydrazine-4-methyl-6-oxypyrimidine, has been described by Pellizzari and Roncagliolo.[3]

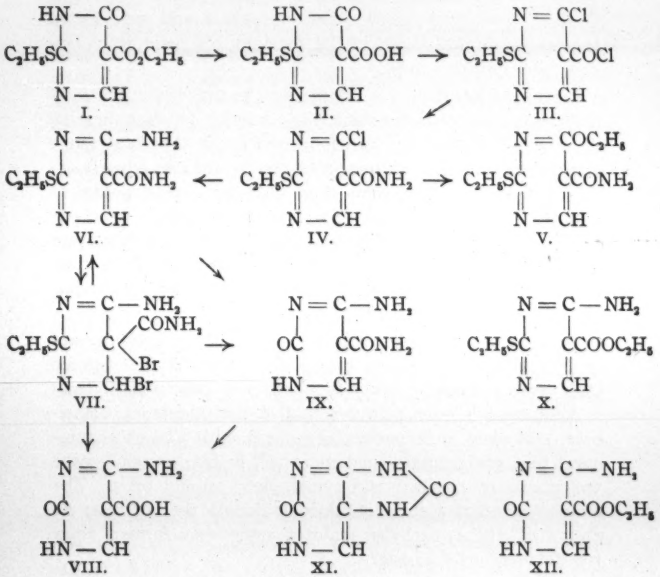
When 2-ethylmercapto-6-aminopyrimidine-5-carboxamide, VI., was boiled with hydrochloric acid it was converted into cytosine-5-carboxylic acid. When given a less energetic treatment with acid, in addition to unaltered material, some cytosine-5-carboxamide along with cytosine-5-carboxylic acid was obtained. This amide was identical with that obtained from the brom product.

The question whether or not the Hofmann rearrangement can be applied in the pyrimidine series still remains to be investigated. A 2-oxygen amide such as cytosine-5-carboxamide, being soluble in alkali and free from sulphur, would be a more promising substance than the mercaptoamide to decide this question. The work will be undertaken as soon

[1] This Journal, **38**, 598 (1907).
[2] Fischer and Dilthey: Ber. d. chem. Ges., **35**, 844.
[3] Gaz. chim. ital., **31**, I, 513.

as a more convenient method of preparing this amide, in quantities sufficient for synthetical purposes, is obtained.

It appears from the above results that the unsaturated condition of the molecule, or the double bond in the 4,5 position, interferes with the Hofmann rearrangement, at least in the case of 2-ethylmercapto-6-aminopyrimidine-5-carboxamide.



EXPERIMENTAL PART.

Ethoxymethylenemalonic Ester, $C_2H_5OCH=C(CO_2C_2H_5)_2$. — Since this ester has proved to be of special importance for pyrimidine syntheses a detailed account is given of our methods of preparing and employing the substance.

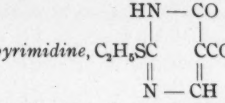
The ester was prepared essentially according to Claisen's directions.[1] We have found that the mixture of malonic ester (100 grams), orthoformic ester (93 grams), acetic anhydride (128 grams), and zinc chloride (7 grams) reacts immediately on gently warming and enough heat is evolved to boil the solution. The mixture was kept boiling for an hour, then all that distilled up to 110° at ordinary pressure (the bulb of the thermometer being *in the solution*) was removed. The distillate usually weighed about 52 grams and represented the ethyl acetate formed in the reaction. A little anhydride was usually carried over.

It was found that it is unnecessary to boil the above mixture for 11–12 hours, as stated by Claisen. In fact, heating the flask attached to a return condenser in an oil bath at 120°–130°, or so that the mixture boiled gently, for two more hours was sufficient. There were then obtained 14–15 grams of impure ethyl acetate or about 67 grams in all, which is 60 per cent of the calculated. The calculated yield of ethyl acetate is 110 grams. Boiling for a longer time gave very little more ethyl acetate. A portion of the ethyl acetate, however, probably remains behind in the mixture.

The mixture was then distilled at 15–20 mm. pressure and in almost every case 60–62 grams of ethoxymethylenemalonic ester were obtained. Sixty-two grams of ethoxymethylenemalonic ester is only 45.9 per cent of the calculated. However, this yield is in perfect agreement with Claisen's statement that 500 grams of malonic ester, in five portions, gave 307 grams of distilled ethoxymethylenemalonic ester. The amount of ethyl acetate obtained indicated that at least 81 grams of ethoxymethylenemalonic ester was formed from 100 grams of malonic ester.

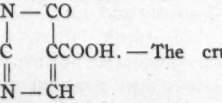
We have now found that it is not necessary or even advisable, on account of loss, to prepare pure distilled ethoxymethylenemalonic ester for the present condensation. In fact, a much better yield of condensation product is obtained if the oil is not distilled.

[1] Ann. Chem. (Liebig), **297**, 75 (1897).

Preparation of the Potassium Salt of 2-Ethylmercapto-5-carbethoxy-6-oxypyrimidine,  .—In one experiment, after heating the above mixture at ordinary pressure for three hours, distilling the ethyl acetate as stated, and filtering from zinc chloride, everything was then removed that distilled below 117° at about 20 mm. pressure. The residue in this case weighed 105 grams. This was added to 100 grams of the addition product of ethyl bromide and thiourea dissolved in 100 cc. of water. To this cold solution 60 grams of potassium hydroxide in 120 cc. of water were slowly added and the mixture was not allowed to warm. The solution soon became semisolid from the separation of the potassium salt. It was found that more separated on standing. The mixture was allowed to stand for two days at ordinary temperature. The salt which separated then, on filtering and drying, weighed 113 grams, or nearly twice as much crude salt as was obtained from 100 grams malonic ester in our previous work.

In another experiment everything was removed that distilled below 120° at 25 mm. pressure. The remaining oil weighed 100 grams. When this was condensed with 2-ethylpseudothiourea as above, the weight of the potassium salt, after washing with a little alcohol, was 91 grams.

The above yields, of course, do not represent the weights of pure substances, they merely serve to give a general idea. We conclude, however, that the above process yields almost 75 per cent of the calculated of ethoxymethylenemalonic ester; 105 grams is 77.7 per cent of the calculated.

Preparation of 2-Ethylmercapto-6-oxypyrimidine-5-carboxylic Acid,  .—The crude potassium salt of 2-ethylmercapto-5-carbethoxy-6-oxypyrimidine was saponified

by warming with potassium hydroxide. For about 27–30 grams of this salt 15 grams of potassium hydroxide were used. If a smaller proportion of alkali was used the saponification was liable to be less smooth.

The potassium salt was dissolved in alcohol, the alkali in water added, and the solution evaporated to dryness on the steam bath. The residue was taken up in water and evaporated again, when the saponification was usually found to be complete. The acid was then precipitated from the filtered aqueous solution of this residue by means of hydrochloric acid. It separated in an almost pure condition as shown by its melting point (near 167°). Care must be taken not to add too much hydrochloric acid since the mercapto acid is soluble in a certain excess. On the other hand, if the solution is not made distinctly acid, the potassium salt of 2-ethylmercapto-6-oxypyrimidine-5-carboxylic acid is obtained. This melts with effervescence above 250°. If the precipitate melts near 131° the saponification is not complete.

Twenty-seven grams of the washed potassium salt gave 17 grams of 2-ethylmercapto-6-oxypyrimidine-5-carboxylic acid. The same quantity of salt in another experiment gave 18 grams of acid. This is 88.6 per cent of the calculated.

The Acid Chloride of 2-Ethylmercapto-6-chlorpyrimidine-5-carboxylic Acid, $C_2H_5SC(N=CCl)(CCOCl)(N-CH)$.—The best yield of this compound was obtained when perfectly dry 2-ethylmercapto-oxypyrimidine-5-carboxylic acid and phosphorus oxychloride were mixed in the proportion of 5 cc. of the chloride to 1 gram of the acid and then heated, with a return condenser, in an oil bath. The temperature of the bath was kept at 130°–150° or so that the solution boiled quietly and until hydrogen chloride was no longer evolved. This required from 2 to 3 hours, although solution took place soon after heating. Most of the phosphorus oxychloride was then removed under reduced pressure at 100° and the residue, a thick oil, was poured upon

cracked ice. It then changed to a granular solid. The yield of crude chloride was 85 per cent of the calculated. To prepare the amide of 2-ethylmercapto-6-aminopyrimidine-5-carboxylic acid, instead of pouring on ice, it is poured directly into concentrated ammonia (see below). The yield of crude amide is then almost quantitative. In another experiment 18 grams of the acid and 45 cc. of phosphorus oxychloride were boiled on a sand bath. Under these conditions the material was completely decomposed. The yield of this chloride may vary decidedly under slight changes of conditions.

This acid chloride was very soluble in cold benzene, ether, and ligroin. It was purified for analysis by dissolving in ligroin and evaporating at the temperature of the room. Radiating clusters of colorless prisms were formed, which melted to a clear oil at 38°–40°. Analysis:

	Calculated for $C_7H_6ON_2Cl_2S$.	Found.
N	11.81	11.76

The Amide of 2-Ethylmercapto-6-chlorpyrimidine-5-carboxylic Acid, $C_2H_5SC(N{=}CCl){-}CCONH_2{=}N{-}CH$.—This is the first product obtained by the action of aqueous ammonia on the above acid chloride. When the acid chloride was added to cold concentrated aqueous ammonia, an immediate reaction took place with the liberation of heat and a white powder was formed. This was washed with water to remove ammonium chloride. When dried it was found to be moderately soluble in hot alcohol, benzene, and toluene, and difficultly soluble in boiling ether. It crystallized from benzene in long, hairlike crystals which formed a fibrous mat. It then melted sharply to a colorless oil at 134° and the yield was almost quantitative. Analysis:

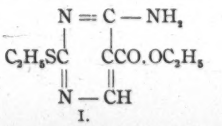
	Calculated for $C_7H_8ON_3ClS$.	Found.	
		I.	II.
N	19.31	19.36	19.24

When this chloramido compound was first obtained it became a question which chlorine atom had been replaced in the above

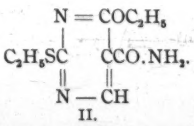
acid chloride. In order to decide this point, two grams of the chloramido compound were dissolved in absolute alcohol and added to a solution of 0.25 gram of sodium, also in alcohol. An immediate precipitate resulted and heat was liberated. On cooling, a mass of well defined prisms separated. The sodium chloride was removed by washing with water. The residue was then found to be insoluble in hot water but it crystallized from alcohol, in which it was moderately soluble. The substance melted at 134°, the same temperature as the chloramido compound. However, it was free from chlorine and when mixed with the chloramido compound the melting point of the mixture was lowered about 30°. The yield and nitrogen determinations agreed with the calculated for an ethoxy derivative.

	Calculated for $C_9H_{13}O_3N_3S$.	Found. I.	Found. II.
N	18.50	18.54	18.45

This ethoxy derivative should have the structure represented by one of the following formulas:

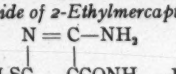


I.



II.

The first of these two compounds has already been described by us in our twenty-sixth paper, p. 597. It melts at 102°. To the ethoxy compound now obtained, melting at 134°, is therefore assigned the structure represented by formula II. It follows that the chloride from which it was prepared has the chlorine atom in the 6 position and is the amide of 2-ethylmercapto-6-chlorpyrimidine-5-carboxylic acid.

The Amide of 2-Ethylmercapto-6-aminopyrimidine-5-carboxylic Acid,  .—If the acid chloride of 2-ethylmercapto-6-chlorpyrimidine-5-carboxylic acid is warmed with

concentrated ammonia both chlorine atoms are replaced by the amino group and the amide of 2-ethylmercapto-6-amino-pyrimidine-5-carboxylic acid is formed. We have prepared this compound by slowly adding the acid chloride (it reacts vigorously) to a considerable excess of aqueous ammonia in an evaporating dish and then warming on the steam bath. Not very much of the aminoamide thus formed dissolves in the hot ammonia nor is it soluble to any great extent in boiling water. It should be warmed until the insoluble material melts above 200°. When purified by crystallizing from alcohol, in which it is moderately soluble, it separates in rectangular crystals, little stout prisms which separate slowly and melt to an oil at 218°–219°. It is soluble in glacial acetic acid but insoluble in alkali. The yield of crude product melting at 214° is almost quantitative. Thirteen grams of mercapto acid gave 12 grams of this amide. Analysis:

	Calculated for $C_7H_{10}ON_4S$.	Found. I.	Found. II.
N	28.28	27.99	27.91

Action of Hydrochloric Acid.—When this amide is digested on the steam bath with concentrated hydrochloric acid ethyl mercaptan is evolved and the amido group attached to the carboxyl radical in position 5 is removed. If the resulting solution is allowed to cool slowly the *hydrochloride of cytosine-5-carboxylic acid* separates in the form of large prisms which contain one molecule of water and melt sharply at 276° to a brown oil and with some effervescence. This salt was identified by its properties previously described and by the following analyses:

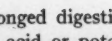
	Calculated for $C_5H_5O_3N_3.HCl.H_2O$.	Found.
H_2O	8.59	8.75

The water determination was made by heating the substance at 120°–130°. A nitrogen determination then gave the following results:

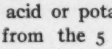
	Calculated for $C_5H_5O_3N_3.HCl$.	Found.
N	21.92	22.21

Prolonged digestion of the aminoamide with dilute hydrochloric acid or potassium hydroxide also removed the amido group from the 5 position. For a description of the less energetic action of hydrochloric acid on this amide see under cytosine-5-carboxamide. It did not dissolve in a solution of potassium hypobromite (16 grams of bromine in 280 cc. of 10 per cent alkali).

The Action of Dry Bromine: The Dibrom Addition Product of 2-Ethylmercapto-6-amino-5-carboxamide,



$C_2H_5SC\quad CCONH_2Br_2$.—This compound is formed by adding



the amide directly to liquid bromine. They unite with evolution of considerable heat. Eight grams of finely powdered and carefully dried amide were added in small portions, best through a sieve to prevent the formation of lumps, to about 15 cc. of dry bromine. The amide then dissolved at once in the bromine. When all of the amide had been added the excess of bromine was allowed to evaporate at the temperature of the room. This treatment left a yellow, crystalline crust that was powdered in a mortar in order to liberate the inclosed bromine. The yield at this point was quantitative if care was taken to exclude moisture; otherwise some decomposition took place. Analyses of the air-dried material gave the following results:

	Calculated for $C_7H_{10}ON_4Br_2S$.	Found. I.	Found. II.
N	15.64	15.50	15.91

When the dibrom addition product was warmed with water unaltered amide was obtained.

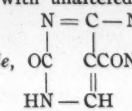
Action of Alkali.—The unaltered amide was also obtained when this material was treated with alkali. One gram of the addition product was added to 10 cc. of water containing 1 gram of potassium hydroxide. Only a small portion dissolved even after heating on the steam bath. On filtering and acidifying the filtrate with acetic acid a very small amount of material not soluble in ammonia was precipitated. This had all the

properties of cytosine-5-carboxamide. Its hydrochloric acid solution gave a precipitate with nitric acid.

Behavior on Heating.—When the dibrom addition product was heated at 120°–135° a point was finally reached where the evolution of hydrogen bromide seemed to stop or proceed more slowly. The brownish white material then agreed in weight with the loss of about one molecular proportion of hydrogen bromide. There was a loss from 13.5 grams of the addition product of 3.3 grams while the calculated loss is 3.1 grams. Nitrogen determinations gave the following results:

	Calculated for $C_7H_9ON_4BrS$.	Found. I.	II.
N	20.21	20.47	20.76

This product had an odor similar to that of the sulphur chlorides. It dissolved only partially in hot water, alcohol, ethyl acetate, or amyl acetate, and it behaved like a mixture of more or less decomposed material. It dissolved partially in a solution of sulphur dioxide, and ammonia then precipitated unaltered mercaptoaminoamide. This might result from the hydrobromic acid salt of the amide possibly contained in the mixture, or from unaltered dibrom addition product. A solvent suitable to isolate a monobromamide, if formed here, was not found, and the results obtained on treating the material with alkali would suggest that in spite of the analyses such a compound was not formed. Sometimes the product would dissolve completely in alkali. When dark in color it usually left undissolved amorphous, dark colored decomposition products mixed at times with unaltered mercaptoaminoamide.

Cytosine-5-carboxamide,  . —Ten grams of the brom product obtained by heating the above dibrom addition product of the amide of 2-ethylmercapto-6-amino-pyrimidine-5-carboxylic acid were dissolved in 50 cc. of water containing 10 grams of potassium hydroxide. In this particular experiment complete solution resulted. On acidifying with

acetic acid mercaptan was evolved and a granular precipitate was obtained. This was filtered and dissolved in hot water to which a little hydrochloric acid was added. The hot solution was then made strongly alkaline with ammonia which reprecipitated the base. This treatment removed any cytosine-5-carboxylic acid that was present, the acid being soluble in ammonia. The best yield of aminoamide obtained at this point was about 75 per cent of the theory. It varied considerably. For analysis the aminoamide was purified by recrystallizing from dilute hydrochloric acid. The hydrochloride was then dissolved in water, with the aid of a little hydrochloric acid, and the base reprecipitated with ammonia, washed thoroughly with water and alcohol, and finally dried over calcium chloride. It did not lose water when heated at 120°–130° (Analyses I. II. and III.).

When we first obtained this material attempts were made to determine nitrogen by Kjeldahl's method and it was found that neither the free base nor any of its salts that we tried gave results agreeing with the calculated. The figures invariably came several per cent too low. It appears probable that the failure to obtain correct results by Kjeldahl's method was due to the fact that the weakly basic properties of the compound permitted the escape of material on heating the sulphuric acid solution. This might have escaped unnoticed if we had no theory to check the results.

I. 0.0763 gram of substance gave 24.6 cc. of nitrogen at 22° and 770 mm. pressure.

II. 0.2462 gram of substance gave 0.0877 gram of H_2O and 0.3575 gram of CO_2.

III. 0.1440 gram of substance gave 0.0526 gram of H_2O and 0.2093 gram of CO_2.

	Calculated for $C_4H_4O_2N_4$.	Found. I.	II.	III.	IV.
C	38.96	39.60	39.58	.....	.....
H	3.89	4.00	3.90	.....	.....
N	36.36	.....	.....	37.00	36.38

Cytosine-5-carboxamide was also obtained by the action of concentrated hydrochloric acid on 2-ethylmercapto-6-amino-

pyrimidine-5-carboxamide. One gram of the mercapto-aminoamide was evaporated to dryness with 15 cc. of concentrated hydrochloric acid on the steam bath. The residue was made strongly alkaline with ammonia. This extracted cytosine-5-carboxylic acid and left a mixture which was boiled with alcohol. Unaltered mercaptoaminoamide dissolved. The insoluble material was purified by dissolving in dilute hydrochloric acid and precipitating with an excess of ammonia while the solution was hot. This gave very small, microscopic, lenticular crystals that did not show signs of melting at 310°. When a drop of nitric acid was added to the hydrochloric acid solution a precipitate of the nitrate was formed. (Analysis IV.).

Cytosine-5-carboxamide is almost insoluble in water and the ordinary organic solvents. It dissolves readily in cold alkalies, but it is almost insoluble in ammonia. It does not dissolve in acetic acid. It dissolves in dilute mineral acids and yields beautiful crystalline salts which are dissociated by water. The aminoamide is a weak base. Its solubility in alkali is in accordance with the presence of —NH—CO— groups. The free aminoamide prepared from the brom derivative, when obtained by precipitating from alkaline solution by acetic acid or from the hydrochloric acid solution with ammonia, usually appears to be an amorphous powder. Before purifying it has a more or less brownish color. Its method of separation along the lines of stirring indicates a crystallization but the crystals are too minute to be determined under the microscope.

Action of Concentrated Hydrochloric Acid on Cytosine-5-carboxamide: Cytosine-5-carboxylic Acid.—The amide obtained from the bromamide (0.6 gram) was boiled with 50 cc. of concentrated hydrochloric acid for about five minutes and the resulting solution evaporated to dryness on the steam bath. The residue dissolved completely in ammonia. On acidifying with acetic acid a precipitate of cytosine-5-carboxylic acid was obtained. This decomposed at 256°. Analysis:

	Calculated for $C_5H_5O_2N_3$.	Found.
N	27.09	27.53

This acid was described in a previous paper. It should be added that, like the amide, it is almost insoluble in water and alcohol. It was obtained in an amorphous condition. When precipitated from its solution in ammonia by acetic acid it separates as a gelatinous precipitate which is difficult to filter. It is better to obtain the acid by evaporating the ammonia solution. It then separates as a finely divided powder which is readily filtered. It is also readily identified by its decomposing point, 256°, and its crystalline hydrochloride which contains 1 molecule of water of crystallization.

Cytosine-5-carboxamide Hydrochloride, $C_5H_6O_2N_4.HCl$.—The base dissolved in moderate quantities in hot dilute hydrochloric acid. On cooling, the hydrochloride separated almost completely in clusters of colorless, stout, pointed prisms which did not have a definite melting point. They did not lose weight on heating at 110°, when dried over calcium chloride. When heated at 140°–150° they lost their lustre, hydrochloric acid being slowly given off. The salt was dried at 110° for analysis:

I. 0.0739 gram of substance gave 19.0 cc. of N at 22° and 768 mm.

II. 0.0791 gram of substance gave 0.0607 gram of AgCl.

	Calculated for $C_5H_6O_2N_4.HCl$.	Found. I.	II.
N	29.40	29.42	.....
Cl	18.64	.....	18.97

Cytosine-5-carboxamide Nitrate, $C_5H_6O_2N_4.HNO_3$.—This is the most difficultly soluble and characteristic salt. It is formed not only by dissolving the amide in hot nitric acid but also on adding nitric acid to solutions of other salts of the base.

Cytosine-5-carboxamide dissolved very slightly in hot nitric acid. On cooling, the nitrate separated in beautiful truncated octahedrons. When nitric acid was added to a solution of the base in dilute hydrochloric acid an immediate crystalline precipitate of the nitrate came down. It then formed tufts of short hairs, which did not decompose at 300°, although they sublimed slowly below that temperature. They did not lose weight on heating at 115°. Analysis:

	Calculated for $C_5H_6O_2N_4.HNO_3$.	Found.
N	32.26	32.05

Cytosine-5-carboxamide Sulphate, $(C_5H_6O_2N_4)_2H_2SO_4$.—A solution of the amide was made in hot water containing about 10 per cent sulphuric acid. The sulphate separated, on cooling, in scales with a pearly lustre. They did not melt at 300° and no loss in weight took place on heating at 110°. Analysis:

0.0653 gram of substance gave 0.0386 gram $BaSO_4$.

	Calculated for $(C_5H_6O_2N_4)_2H_2SO_4$.	Found.
S	7.88	8.11

Cytosine-5-carboxamide Picrate, $C_5H_6O_2N_4.C_6H_2(NO_2)_3OH$.—The amide was dissolved in hot water to which a few drops of hydrochloric acid had been added. On adding an aqueous solution of picric acid an immediate precipitate was formed. This crystallized from an aqueous solution of picric acid. It dissociates in water into small, yellow prisms that began to decompose at about 260°–270°. The salt was found to be anhydrous.

I. 0.0531 gram of substance gave 11.6 cc. of N at 20° and 771 mm.

II. 0.0967 gram of substance gave 21.2 cc. of N at 18° and 771 mm.

	Calculated for $C_{11}H_9O_8N_7$.	Found.	
		I.	II.
N	25.59	25.34	25.64

The papers on pyrimidines which have been published from this laboratory, under the direction of Dr. Treat B. Johnson and the writer, have appeared in two journals. Some of these papers have not been numbered and one has received an incorrect number, there being two twenty-first papers. For the sake of reference a complete list of these papers up to the end of the year 1907 is now given. The papers are arranged and numbered in the order in which they appeared. The numbers correspond with those already given in the literature, with the exception of the paper which has been incorrectly numbered. By referring to this paper as the twenty-

fourth the order then remains otherwise undisturbed. The following is a complete list up to the end of the year 1907.

1. Researches on the Cycloamidines: Pyrimidine Derivatives. H. L. Wheeler: THIS JOURNAL, **20**, 481 (1898).

2. On Some Condensation Products of the Pseudothioureas: Synthesis of Uracil, Thymine, and Similar Compounds. Henry L. Wheeler and Henry F. Merriam: THIS JOURNAL, **29**, 478 (1903).

3. Syntheses of Aminooxypyrimidines having the Composition of Cytosine: 2-Amino-6-oxypyrimidine and 2-Oxy-6-aminopyrimidine. Henry L. Wheeler and Treat B. Johnson: THIS JOURNAL, **29**, 492 (1903).

4. On Cytosine or 2-Oxy-6-aminopyrimidine from Triticonucleic Acid. Henry L. Wheeler and Treat B. Johnson: THIS JOURNAL, **29**, 505 (1903).

5. 5-Methylcytosine. Henry L. Wheeler and Treat B. Johnson: THIS JOURNAL, **31**, 591 (1904).

6. Synthesis of 2-Amino-5-methyl-6-oxypyrimidine. Treat B. Johnson and Samuel H. Clapp: THIS JOURNAL, **32**, 130 (1904).

7. 2-Oxy-4,6-diaminopyrimidine. Henry L. Wheeler and George S. Jamieson: THIS JOURNAL, **32**, 342 (1904).

8. The Structure of Some Substitution Products. Henry L. Wheeler and H. Stanley Bristol: THIS JOURNAL, **33**, 437 (1905).

9. The Action of Potassium Thiocyanate upon Some Imidechlorides. Henry L. Wheeler and H. Stanley Bristol: THIS JOURNAL, **33**, 448 (1905).

10. The Action of Aqueous and Alcoholic Ammonia and Aniline on Some Halogen- and Mercaptopyrimidines. Treat B. Johnson and Carl O. Johns: THIS JOURNAL, **34**, 176 (1905).

11. 2-Ethylmercapto-5-amino-6-oxypyrimidine. Treat B. Johnson: THIS JOURNAL, **34**, 191 (1905).

12. 2,5-Diamino-6-oxypyrimidine. Treat B. Johnson and Carl O. Johns: THIS JOURNAL, **34**, 554 (1905).

13. Some Iodopyrimidines: 5-Iodocytosine. Treat B. Johnson and Carl O. Johns: *J. Biol. Chem.*, **1**, 305 (1906).

14. On Methods of Synthesizing Isobarbituric Acid and 5-Oxycytosine. Treat B. Johnson and Elmer V. McCollum: *J. Biol. Chem.*, **1**, 437 (1906).

15. The Action of Potassium Thiocyanate upon Imidechlorides. Treat B. Johnson and Elmer V. McCollum: THIS JOURNAL, **36**, 136 (1906).

16. The Formation of Purines from Ureapyrimidines. Treat B. Johnson and Elmer V. McCollum: THIS JOURNAL, **36**, 149 (1906).

17. 5-Nitrocytosine and its Reduction to 2-Oxy-5,6-diaminopyrimidine. Treat B. Johnson, Carl O. Johns, and Frederick W. Heyl: THIS JOURNAL, **36**, 160 (1906).

18. 5-Ethylcytosine. Treat B. Johnson and George A. Menge: *J. Biol. Chem.*, **2**, 105 (1906).

19. Synthesis of Uracil-5-carboxylic Acid. Henry L. Wheeler, Treat B. Johnson, and Carl O. Johns: THIS JOURNAL, **37**, 392 (1907).

20. Some Condensation Products of a Substituted Pseudothiourea:

Synthesis of 1-Methyluracil. Treat B. Johnson and F. W. Heyl: THIS JOURNAL, **37**, 628 (1907).

21. On a Color Test for Uracil and Cytosine. Henry L. Wheeler and Treat B. Johnson: *J. Biol. Chem.*, **3**, 183 (1907).

22. On Some Salts of Cytosine, Isocytosine, 6-Aminopyrimidine, and 6-Oxypyrimidine. Henry L. Wheeler: *J. Biol. Chem.*, **3**, 285 (1907).

23. Uracil-4-carboxylic Acid. Henry L. Wheeler: THIS JOURNAL, **38**, 358 (1907).

24. The Action of Methyl Iodide on 2-Anilino-6-oxypyrimidine and the Synthesis of 2-Anilinopyrimidine. Treat B. Johnson and Frederick W. Heyl: THIS JOURNAL, **38**, 237 (1907).

25. Synthesis of Thymine-4-carboxylic Acid. Treat B. Johnson: *J. Biol. Chem.*, **3**, 299 (1907).

26. Synthesis of Cytosine-5-carboxylic Acid. Henry L. Wheeler and Carl O. Johns: THIS JOURNAL, **38**, 594 (1907).

27 Synthesis of Thymine-5'-carboxylic Acid. (Uracil-5-acetic Acid). Treat B. Johnson and Carl Frank Speh: THIS JOURNAL, **38**, 602 (1907).

28. Synthesis of 4-Methyluracil-5-acetic Acid. Treat B. Johnson and Frederick W. Heyl: THIS JOURNAL, **38**, 659 (1907).

NEW HAVEN, CONN.,
March, 1908.

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PEROXIDASE ACCELERATORS AND THEIR POSSIBLE SIGNIFICANCE FOR BIOLOGICAL OXIDATIONS.

BY J. H. KASTLE.

As is well known, many animal and vegetable secretions and many active extracts of living tissues possess the power of inducing the oxidation of various oxidizable substances by means of hydrogen peroxide. Such oxygen carriers are held by many chemists[1] to properly belong to the group of catalysts known as enzymes or unorganized ferments and have been called peroxidases[2] to distinguish them from the oxidases proper. Among various animal secretions, raw milk has been found to give the peroxidase reaction with hydrogen peroxide and various peroxidase reagents, among which may be mentioned guaiacum, potassium iodide and starch, paraphenylenediamine, ortol, paradiethylphenylenediamine, ursol, D., guaiacol, amidol, etc. These reactions with milk have been

[1] Bach: Ber. d. chem. Ges., **37**, 1342–1348, and 2434–2440 (1904).
[2] Linossier: Compt. rend. soc. biol., **5**, 373 (1898).

turned to practical account as affording a simple means of distinguishing between raw milk and that which has been sterilized by heat.[1] Unfortunately, however, as has been pointed out by Rubner,[2] cows' milk is liable to considerable variation in peroxidase activity and hence the general usefulness of the peroxidase reaction of milk, in its original form, as a means of distinguishing between raw and cooked milk, is considerably impaired. In this connection, however, it has recently been pointed out by Kastle and Porch[3] that the peroxidase reaction of milk can be greatly intensified through the action of certain substances, especially certain of the phenols,[4] and that through the use of these substances along with the ordinary peroxidase reagents, such as guaiacum, paraphenylenediamine, and phenolphthalin, the peroxidase reaction of milk becomes a safe and valuable criterion for distinguishing between raw milk and that which has been sterilized by heat.

The results of some of our experiments with milk will serve to make this clear. The peroxidase activity of fresh specimens of cows' milk, obtained directly from the herd, and the influence thereupon of a mixture of the cresols ("Trikresol"[5]) and β-naphthol was determined, using phenolphthalin as the substance to be oxidized, in the following manner:

Tubes were prepared containing 2 cc. of a fresh specimen of cows' milk and 1 cc. of water or a 1 per cent solution of trikresol or β-naphthol, and to these was then added 1 cc. of a solution of phenolphthalin containing 0.320 milligram of the compound in the form of the neutral sodium salt, and 0.1 cc. 0.05 N hydrogen peroxide. The tubes containing these several substances were then allowed to stand for 15 minutes at ordinary temperature (about 20° C.), at the end of which time 2 cc. of 0.1 N sodium hydroxide were added to each tube. On

[1] The literature of this subject is very extensive. See: "The Chemistry of Milk," Article 10, Bulletin No. 41, Hyg. Lab., U. S. Pub. Health and Mar. Hosp. Serv., Wash., pp. 309–417.

[2] Hygienische Rundschau, **5**, 1021–1022 (1895).

[3] See: "The Peroxidase Reaction of Milk," J. Biol. Chem., **5**, 301-320 (1908).

[4] The term "phenol" is here used in its general sense.

[5] "Trikresol" is an antiseptic containing the three cresols in approximately equal proportions.

addition of the alkali the contents of the several tubes become pink to deep purplish red in color from the phenolphthalein resulting from the oxidation, and the intensity of the red color thus produced is a measure of the quantity of phenolphthalein formed and therefore a measure also of the peroxidase activity of any given specimen of milk towards phenolphthalin. In order to make these measurements as nearly quantitative as possible, the quantity of phenolphthalein produced in each observation was determined by colorimetric comparison with

Table I.—Showing the Influence of Trikresol on the Peroxidase Activity of Cows' Milk towards Phenolphthalin.

(*January 21, 1908.*)

Number of specimen of milk.	Phenolphthalein formed, in milligrams.		Per cent of phenolphthalin oxidized.	
	Milk + water.	Milk + trikresol.	Milk + water.	Milk + trikresol.
1	0.020	0.050	6.29	15.72
2	0.064	0.045	20.13	14.15
3	0.025	0.159	7.86	50.00
4	0.020	0.038	6.29	11.95
5	0.024	0.054	7.55	16.98
6	0.057	0.159	17.92	50.00
7	0.057	0.127	17.92	39.94
8	0.057	0.191	17.92	60.06
9	0.010	0.080	3.14	25.16
10	0.003	0.095	0.94	29.87
11	0.048	0.159	15.09	50.00
12	0.048	0.127	15.09	39.94
13	0.080	0.127	25.16	39.94
14	0.010	0.048	3.14	15.09
15	0.020	0.032	6.29	10.06
16	0.032	0.064	10.06	20.13
17	0.020	0.159	6.29	50.00
18	0.032	0.095	10.06	29.87
19	0.025	0.127	7.86	39.94
20	0.020	0.032	6.29	10.06
21	0.013	0.048	4.09	15.09
22	0.025	0.057	7.86	17.92
23	0.010	0.048	3.14	15.09
24	0.024	0.064	7.55	20.13
25	0.048	0.048	15.09	15.09
26	0.048	0.095	15.09	29.87
Average.....			10.16	28.16

Table II.—Showing the Influence of β-Naphthol on the Peroxidase Activity of Cows' Milk towards Phenolphthalin.
(January 22, 1908.)

Number of specimen of milk.	Phenolphthalein formed, in milligrams.		Per cent of phenolphthalin oxidized.	
	Milk + water.	Milk + β-naphthol.	Milk + water.	Milk + β-naphthol.
1	0.006	0.254	1.89	79.87
2	0.064	0.223	20.13	70.13
3	0.032	0.318	10.06	100.00
4	0.020	0.223	6.29	70.13
5	0.016	0.191	5.03	60.06
6	0.095	0.318	29.87	100.00
7	0.095	0.318	29.87	100.00
8	0.095	0.318	29.87	100.00
9	0.010	0.095	3.14	29.87
10	0.020	0.191	6.29	60.06
11	0.016	0.254	5.03	79.87
12	0.095	0.254	29.87	79.87
13	0.095	0.223	29.87	70.13
14	Trace	0.318	.....	100.00
15	0.025	0.318	7.86	100.00
16	Trace	0.318	.....	100.00
17	"	0.191	.....	60.06
18	"	0.318	.....	100.00
19	"	0.254	.....	79.87
20	0.010	0.159	3.14	50.00
21	0.013	0.223	4.09	70.13
22	0.013	0.223	4.09	70.13
23	0.000	0.025	0.00	7.86
24	Trace	0.159	.....	50.00
25	0.006	0.318	1.89	100.00
26	0.000	0.223	0.00	70.13
27	0.032	0.254	10.06	79.87
	Average.....		8.83	71.74

a series of standards containing known amounts of phenolphthalein, milk, alkali, and sufficient quantities of water to make the dilution the same in all cases, *viz.*, 6.1 cc. On account of the great turbidity of solutions containing milk, it is evident that such color comparisons are only approximate. It has been my experience, however, that more exact color comparisons can be made by this method than one would ordinarily suppose to be possible, and that, all things con-

sidered, the results of the observations recorded in the tables closely approximate the actual quantities of phenolphthalein formed. In Table I. are given the results of our observations on the oxidation of phenolphthalin by milk and hydrogen peroxide, with and without trikresol; and in Table II., the results obtained with and without β-naphthol.

It is evident from these results that the oxidation of phenolphthalin by the peroxidase of cows' milk and hydrogen peroxide is greatly accelerated by small amounts of a mixture of the three cresols and by β-naphthol. The degree of acceleration brought about by trikresol and β-naphthol, respectively, varies greatly, depending on the individual nature of the milk and on factors which at present are not clearly understood. With some specimens of fresh cows' milk, over thirty times as much phenolphthalin is oxidized in the presence of trikresol as is oxidized by the milk and hydrogen peroxide alone; and on an average, three times as much. Similarly, the peroxidase activity of certain specimens of milk is increased a hundredfold, at least, by small amounts of β-naphthol; and on an average, eight times as much phenolphthalin is oxidized when β-naphthol is present as is oxidized by the milk and hydrogen peroxide alone; and towards guaiacum and paraphenylenediamine, the peroxidase activity of fresh specimens of cows' milk is also greatly intensified by small amounts of phenol, the cresols, β-naphthol, etc. As a matter of fact, the delicacy of the peroxidase reaction of milk is so greatly intensified by means of such peroxidase accelerators that by means of the test in its present form it has been found possible to readily distinguish between raw and heated milks, using single drops of milk and of the several reagents required, and through the use of these accelerators the peroxidase reaction of milk has been brought within the range of the most delicate chemical reactions (by the wet method). The question, therefore, naturally suggests itself: Is the accelerating action of such substances as phenol, the cresols, and β-naphthol, confined to the peroxidase of milk or is the peroxidase activity of other secretions and tissues intensified by these and similar substances? Without entering into all of the details of the experiments, it may be said in general that the activity of the

peroxidases from whatever source thus far obtained has been found to be greatly increased by phenol, the cresols, and β-naphthol, so that the effect of such substances on the progress of peroxidase oxidations seems to be characteristic of the peroxidases as a class and by no means confined to the peroxidase of milk. It should be observed in this connection, however, that the amounts of these several substances required to show the greatest acceleration of the oxidation in any particular case depends, to some extent at least, on the nature and activity of the particular peroxidase under investigation. For example, with the powerful peroxidase of the root of the horse-radish the best results have been obtained by working with very small amounts, both of the peroxidase and the accelerator. Indeed, it has been found that, as a rule, the more active the peroxidase the smaller the quantity of accelerator required to increase the oxidation above the normal. In order to determine the effect of various substances on the activity of the peroxidase of horse-radish root, tubes were prepared containing the following substances: 1 cc. of water, 0.05 cc. of water or of a 1 per cent solution of the substance to be tested, 0.05 cc. of an active aqueous extract of the fresh root of the horse-radish, 1 cc. phenolphthalin containing 0.320 milligram of the compound in the form of the neutral sodium salt, and 0.1 cc. 0.05 N hydrogen peroxide. In the control experiments, the tubes contained precisely the same amounts of all these substances, the only difference being that in all of the control experiments the aqueous extract of horse-radish used had been boiled for a few moments immediately before the experiments were carried out. The tubes, including the controls, were then allowed to stand at room temperature (17° to 19° C.) for 15 minutes, at the end of which time, 1 cc. of 0.1 N sodium hydroxide was added to each tube, the color was then noted, and the amount of phenolphthalein formed was determined by color comparisons with a standard containing 0.0318 milligram phenolphthalein and 1 cc. 0.1 N sodium hydroxide, at a total dilution of 3.2 cc. The results obtained with the active peroxidase of horse-radish, together with those of the control experiments, are given in Table III.

Table III.—Showing the Influence of Various Substances on the Peroxidase Activity of an Aqueous Extract of Horse-Radish Root.

(*a*) Color produced on addition of 1 cc. 0.1 N sodium hydroxide.
(*b*) Colorimeter readings obtained on comparison with standard phenolphthalein containing 0.0318 milligram phenolphthalein + 1 cc. 0.1 N sodium hydroxide at a total dilution of 3.2 cc., set at one division on the colorimeter scale.
(*c*) Quantity of phenolphthalein formed in the oxidation, in milligrams.
(*d*) Amounts of phenolphthalin oxidized, in milligrams.
(*e*) Percentage of phenolphthalin oxidized.
(*f*) Results of the control experiments.

Number of experiment.	Name of substance.	(*a*).	(*b*).	(*c*).	(*d*).	(*e*).	(*f*).
1	Water	Pink	5.0	0.0063	0.00634	1.98	Faint pink[1]
2	Phenol	Red	0.6	0.0530	0.05332	16.50	Trace of pink
3	Resorcin	Red	0.5	0.0636	0.06398	19.96	"
4	Tannin	Yellowish-brown	...	.....	.....	.....	Yellowish brown
5	β-Naphthol	Red[2]	0.3	0.1060	0.10664	33.32	Faint pink
6	*o*-Cresol	Deep pink	1.0	0.0318	0.03200	10.00	Trace of pink
7	*m*-Cresol	Red	0.5	0.0636	0.06398	19.96	"
8	*p*-Cresol	Red	0.4	0.0795	0.07998	25.00	"
9	Eugenol	Faint pink	6.0	0.0053	0.00533	1.66	"
10	Thymol	Pink	5.0	0.0063	0.00634	1.98	"
11	Guaiacol	Pink	5.0	0.0063	0.00634	1.98	"
12	Benzoic acid	Deep pink	2.5	0.0126	0.01268	3.96	"
13	Salicylic acid	Pink	2.5	0.0126	0.01268	3.96	"
14	Triiodophenol	Pink	4.0	0.0080	0.00805	2.50	"
15	Trikresol	Red	0.5	0.0636	0.06398	19.96	"

[1] The several amounts of phenolphthalein formed in the control experiments were too small to be determined colorimetrically.

[2] The solutions containing horse-radish peroxidase and β-naphthol frequently became pink in color even before the addition of alkali. This change of color, before the addition of alkali, is due to the fact that the phenolphthalin undergoing oxidation in these experiments is present in the solution in the form of a neutral sodium salt. On oxidation, however, it is converted into the monosodium salt of phenolphthalein which therefore should exhibit, to a certain extent at least, the characteristic color of phenolphthalein in alkali. As the color of phenolphthalein in alkali depends, within certain limits, on the concentration of the alkali, the color is greatly intensified by the addition of sodium hydroxide.

It is evident from the results given in Table III. that thymol, eugenol, tannin, and guaiacol are practically without effect on the oxidation of phenolphthalin by horse-radish peroxidase and hydrogen peroxide. Triiodophenol and benzoic and salicylic acids slightly accelerate the oxidation. It will be observed, however, that in the presence of small amounts of phenol, the three cresols, acting singly or together, resorcin, and β-naphthol, from five to fifteen times as much phenolphthalin is oxidized by horse-radish peroxidase and hydrogen peroxide as is oxidized by the peroxidase and hydrogen peroxide alone. Instances of the acceleration of peroxidase oxidations by means of substances of the phenol type might be multiplied at length, for the reason that by means of paraphenylenediamine and phenolphthalin, evidences of such acceleration have been obtained with peroxidases from every source thus far examined, including the peroxidase of the potato, carrot, and other plants, and that of saliva, certain abnormal urines, pus, etc., etc. It is unnecessary, however, in the present discussion to give the results of all these experiments in detail. For the sake of completeness, however, a few of the experiments with fresh extract of malt and saliva are here submitted.

In order to determine the effect of phenol on the peroxidase of a fresh extract of malt, the following tubes were prepared:

(1) 1 cc. phenolphthalin, 0.1 cc. 0.05 N hydrogen peroxide, and 2.05 cc. water.

(2) 1 cc. " 0.1 cc. 0.05 N hydrogen peroxide, 0.05 cc. 1 per cent phenol, and 2 cc. water.

(3) 1 cc. " 0.1 cc. 0.05 N hydrogen peroxide, 1 cc. malt, and 1.05 cc. water.

(4) 1 cc. " 0.1 cc. 0.05 N hydrogen peroxide, 1 cc. malt, 1 cc. water, and 0.05 cc. 1 per cent phenol.

(5) 1 cc. " 0.1 cc. 0.05 N hydrogen peroxide, 1 cc. malt (boiled), 1 cc. water, and 0.05 cc. 1 per cent phenol.

These tubes were allowed to stand 15 minutes at 17° C., when 1 cc. of 0.1 N sodium hydroxide was added to each tube. The results are given in Table IV.

Table IV.

(*a*) Color produced on addition of 1 cc. 0.1 N sodium hydroxide.
(*b*) Colorimeter readings obtained on comparison with standard phenolphthalein containing 0.0318 milligram phenolphthalein + 1 cc. 0.1 N sodium hydroxide at a total dilution of 4.15 cc., set at one division on the colorimeter scale.
(*c*) Quantity of phenolphthalein formed in the oxidation, in milligrams.
(*d*) Amounts of phenolphthalin oxidized, in milligrams.
(*e*) Percentage of phenolphthalin oxidized.

Number of experiment.	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)
1	Trace of pink[1]	....	.....	.....	.....
2	"	....	.....	.....	.....
3	Pink	1.00	0.0318	0.032	10.00
4	Red	0.25	0.1272	0.128	40.00
5	Faint yellowish	....	.....	.....	

Using human saliva instead of fresh extract of malt, the conditions otherwise being the same as with malt, the following results were obtained.

Table V.

Number of experiment.	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)
1	Trace of pink[1]	.....	.....	.....	....
2	"	.....	.....	.....	....
3	Faint pink	12.00	0.00265	0.00267	0.83
4	Pink	5.00	0.00636	0.00640	2.00
5	Colorless	.....	.....	.....	....

In Tables VI. and VII. are given the results of further observations on the effect of various substances on the peroxidase activity of malt and human saliva. Tubes were prepared containing 1 cc. water, 1 cc. malt, 1 cc. phenolphthalin, 0.10 cc. 0.05 N hydrogen peroxide, and 0.05 cc. of the solution to be tested or an equal volume of distilled water. These were allowed to remain at room temperature (16°–21° C.) for 65 minutes, at the end of which time 1 cc. 0.1 N sodium hydroxide was added to each tube and the resulting colors

[1] In experiments 1, 2, and 5 the quantities of phenolphthalein formed were too small to be determined colorimetrically.

compared with a standard phenolphthalein solution containing 0.0318 milligram phenolphthalein, 1 cc. of malt, 1 cc. 0.1 N sodium hydroxide, and water sufficient to bring the total volume of the standard to 4.15 cc.

Table VI.—Showing the Influence of Various Substances on the Peroxidase Activity of Fresh Malt Extract.

(*a*) Color produced on addition of 1 cc. 0.1 N sodium hydroxide.
(*b*) Colorimeter readings obtained on comparison with the standard set at one division on the colorimeter scale.
(*c*) Quantity of phenolphthalein formed, in milligrams.
(*d*) Amounts of phenolphthalin oxidized, in milligrams.
(*e*) Percentage of phenolphthalin oxidized.

Number of experiment.	Name of substance.	(*a*).	(*b*).	(*c*).	(*d*).	(*e*).
1	Water	Pink	1.25	0.0254	0.0256	8.00
2	β-Naphthol	Red	0.50	0.0636	0.0640	20.00
3	Phenol	Deep red	0.40	0.0795	0.07998	25.00
4	*o*-Cresol	Deep red	0.40	0.0795	0.07998	25.00
5	*m*-Cresol	Red	0.50	0.0636	0.0640	20.00
6	*p*-Cresol	Deep red	0.40	0.0795	0.07998	25.00
7	Resorcin	Deep pink	0.70	0.0454	0.0457	14.20
8	Thymol	Pink	1.25	0.0254	0.0256	8.00
9	Benzoic acid	Pink	1.30	0.0245	0.0247	7.70
10	Salicylic acid	Pink	1.40	0.0227	0.0228	7.10
11	Tannin	Yellowish brown	....	.....	.....	.....

In the experiments with human saliva, tubes were prepared containing 1 cc. of mixed saliva (human) which had previously been diluted with an equal volume of water and filtered until clear, 1 cc. phenolphthalin, 0.05 cc. of water or of a one per cent solution of the substance to be tested, and 0.10 cc. of 0.05 N hydrogen peroxide. The tubes were then allowed to stand at room temperature (21.5° to 24.5° C.) for 15 minutes, when 1 cc. 0.1 N sodium hydroxide was added to each tube and the colors produced compared with a standard containing 0.0318 milligram of phenolphthalein, 1 cc. 0.1 N sodium hydroxide, 1 cc. of saliva, and water sufficient to make the total volume of the standard equal to 3.15 cc.

Table VII.—Showing the Influence of Various Substances on the Peroxidase Activity of Human Saliva.

(*a*) Color produced on addition of 1 cc. 0.1 N sodium hydroxide.
(*b*) Colorimeter readings obtained on comparison with the standard set at one division on the colorimeter scale.
(*c*) Quantity of phenolphthalein formed, in milligrams.
(*d*) Amounts of phenolphthalin oxidized, in milligrams.
(*e*) Percentage of phenolphthalin oxidized.

Number of experiment.	Name of substance.	(*a*).	(*b*).	(*c*).	(*d*).	(*e*).
1	Water	Trace of pink[1]	3.2	0.0099	0.00996	3.1
2	β-Naphthol	Light pink	1.0	0.0318	0.032	10.00
3	Phenol	Deep pink	3.0	0.0106	0.01066	3.33
4	*o*-Cresol	Light pink	1.6	0.0198	0.0199	6.20
5	*m*-Cresol	Deep pink	1.5	0.0212	0.0213	6.65
6	*p*-Cresol	Deep pink	...	.....	.....	.....
7	Resorcin	Trace of pink	...	.....	.....	....
8	Thymol	"	...	.....	.....	....
9	Benzoic acid	"	...	.....	.....	....
10	Salicylic acid	"	...	.....	.....	....
11	Tannin	Yellowish brown	...	.....	.....	....

It would seem, therefore, that so far as their effect on peroxidase oxidations is concerned, substances may be arranged in three classes:

(1) Those which accelerate the oxidation.
(2) Those which exert little or no influence upon it.
(3) Those which retard it.

To class (1) belong the following substances: Phenol, the cresols, β-naphthol, resorcin, vanillin,[2] etc., all of which have been found to greatly accelerate peroxidase oxidations under certain conditions. In this class of substances might also be included iodine[3] and hydrogen ions,[4] both of which, according to Bach, intensify the action of peroxidase at certain concentrations. Dupouy[5] has made the interesting observation that magnesium sulphate augments the peroxidase activity of saliva, and Gillet[6] has found that the

[1] In experiments 1, 7, 8, 9, 10, and 11, the quantity of phenolphthalein formed was too small to be determined colorimetrically.
[2] M. B. Porch found that vanillin accelerates the peroxidase reaction of milk.
[3] Ber. d. chem. Ges., **40**, 230–235 (1907).
[4] *Ibid.*, **37**, 3785-3800 (1905).
[5] See Dupouy: Thèse, Bordeaux 1899, p. 43.
[6] J. de Physiol. et de Pathol. Gen., **4**, 442 (1902).

peroxidase activity of cows' milk is increased by this salt. Of the various substances whose effect on the peroxidase oxidation has been tested in this laboratory quite a number, including triiodophenol, cane sugar, maltose, formic aldehyde, glycin, malic and tartaric acids, etc., etc., have been found to slightly accelerate the oxidation. Whether these substances properly belong to the class of peroxidase accelerators or to class (2) can only be determined by further investigation.

To class (2) belong thymol, eugenol, guaiacol, benzoic acid, salicylic acid, tannin, and benzyl alcohol.

To class (3) belong, according to Hunger,[1] the tannins and certain sugars; certain substances ordinarily classed among the poisons, such as hydroxylamine, hydrazine, hydrocyanic acid and the cyanides, whose effect on the peroxidase reaction has recently been investigated by Bach;[2] and also acids and alkalies at certain concentrations. Catalase has also been found by Chodat and Pasmanik[3] to retard the oxidation of hydriodic acid by peroxidase and hydrogen peroxide.

It should be observed in this connection, however, that at present it is impossible to adopt any hard and fast classification of substances with regard to their effect on peroxidase oxidations for the reason that a given substance, which under certain conditions and at certain concentrations is without influence on the oxidation, may under other conditions and at other concentrations greatly accelerate it. Such a substance is resorcin, which under the conditions of our experiments has been found to be practically without influence on the peroxidase reaction of milk, whereas it has been found to greatly accelerate the oxidation of phenolphthalin by hydrogen peroxide and the peroxidase of horseradish. Our results show conclusively, however, that certain substances, especially certain compounds of the type of the phenols, do exert a markedly accelerating effect on

[1] Ber. d. botan. Ges., **19**, 374–377.
[2] Ber. d. chem. Ges., **40**, 3185–3191 (1907).
[3] Arch. Sci. Phys. Nat., **23**, 386–393 (1907).

such oxidations as are accomplished by hydrogen peroxide through the aid of a peroxidase. In my opinion, such substances act in the capacity of auxiliary oxygen carriers and are themselves oxidized more or less completely in such processes. That such is the case is indicated by the fact that most of them, at least, when acted on by hydrogen peroxide and a peroxidase exhibit characteristic color changes which other chemists, among them Bertrand,[1] Bourquelot,[2] Gillet,[3] and Bach,[4] have attributed to oxidation; and also by the fact that mixtures containing such substances, together with hydrogen peroxide and an active peroxidase, and which originally possess powerful oxidizing properties, soon lose their oxidizing power when heated or even allowed to stand for a short interval at ordinary temperature.

In the light of our present knowledge concerning the mechanism of oxidations accomplished by hydrogen peroxide under the influence of an oxygen carrier,[5] it seems reasonable to suppose that a peroxidase accelerates oxidations by hydrogen peroxide by reason of the fact that it forms a peroxide of some kind which is less stable than hydrogen peroxide itself and which therefore gives up its oxygen more easily than hydrogen peroxide to any oxidizable substance which may happen to be present.[6] Graphically, these changes may be represented in the following, in which P represents the peroxidase and B the peroxidase reagent:

$$(1)\ P + H_2O_2 \longrightarrow PO_2,$$

and

$$(2)\ PO_2 + 2B = 2BO + P,$$

or

$$(3)\ PO_2 + B = BO + PO.$$

[1] Bull. Soc. Chim. [3], **11**, 717–721 (1894).

[2] Compt. rend., **123**, 315–317 (1896).

[3] J. de Phys. et de Path. Gen., **4**, 450 (1902).

[4] Ber. d. chem. Ges., **40**, 230–235 (1907).

[5] See Loevenhart and Kastle: THIS JOURNAL, **29**, 397–437; and also Kastle and Loevenhart: *Ibid.*, **29**, 563–588 (1903).

[6] See Kastle and Loevenhart: THIS JOURNAL, **26**, 564–566 (1901). This view regarding the mode of action of a peroxidase on hydrogen peroxide has been confirmed by the later work of Bach and Chodat; see Ber. d. chem. Ges., **37**, 1342–1348 (1904).

It is also well known that one peroxide may be formed from another by processes of ordinary double decomposition.[1] Hence it is readily conceivable that under certain conditions the peroxide resulting from the action of the hydrogen peroxide on the peroxidase might convert a second oxidizable compound, *viz.*, the auxiliary oxygen carrier, into a peroxide and that this in turn might accomplish the oxidation of another oxidizable substance, *viz.*, the peroxidase reagent (phenolphthalin, guaiacum, paraphenylenediamine, etc.), more readily than this could be accomplished by the peroxide resulting from the action of the peroxidase itself on the hydrogen peroxide. Graphically, these changes may be represented in the following, in which P represents the peroxidase, A, the auxiliary oxygen carrier, and B, the peroxidase reagent, that is, the substance whose oxidation is really under observation:

$$(1)\ P + H_2O_2 \rightarrow PO_2$$

and

$$(2)\ PO_2 + A = P + AO_2,$$

or

$$(3)\ 2PO_2 + A = 2PO + AO_2,$$

and

$$(4)\ AO_2 + B = AO + BO.$$

This view of the process enables us to understand the function of an auxiliary oxygen carrier in peroxidase oxidations and is capable of practically unlimited extension.

According to Engler[2] and others, hydrogen peroxide may result directly from the autoxidation of various substances containing labile hydrogen atoms, such as indigo white, etc., etc., and also by the hydrolysis of another peroxide, thus:

$$RO_2 + H_2O = RO + H_2O_2.$$

It might therefore be formed under certain conditions from

[1] See Engler and Weisberg: "Vorgänge der Autoxydation," Braunschweig, 1904, p. 31.

[2] *Ibid.*, 30–32.

an oxygenase, and as pointed out by Bach and Chodat,[1] hydrogen peroxide itself may play a definite rôle in the life of the cell. The universal distribution of catalase in living tissues and secretions would also seem to point to the formation of hydrogen peroxide in the respiratory process. According to Loew,[2] the function of catalase is to protect the living organism against the accumulation of hydrogen peroxide within the tissues. There can be no doubt, however, that while it would be largely decomposed into water and oxygen, under these conditions a part of it, at least, would probably be utilized in the oxidation of readily oxidizable substances, as a result of the action of the peroxidases; and in the light of the results described in the foregoing part of this communication, we can readily understand how, if hydrogen peroxide were ever formed in the living cell, it could be turned to immediate account for purposes of active and vigorous oxidation through the combined action of a peroxidase and a peroxidase accelerator. It is also readily conceivable that if certain substances accelerate oxidations accomplished by a peroxidase and hydrogen peroxide, they might also accelerate oxidations accomplished by means of a peroxidase and other peroxides, including the oxygenases. It also seems reasonable to suppose that among the great diversity of readily oxidizable substances met with in living tissues and in animal and plant secretions, a number would be found to greatly accelerate peroxidase oxidations. To take a case in point, Bertrand[3] found such a readily oxidizable substance in the sap of the lac tree, *viz.*, laccol, a substance which, according to this author, possesses the properties of a polyatomic phenol and whose oxidation by molecular oxygen, under the influence of laccase, gives rise to the black pigment of lac varnish. It, therefore, seems probable, in the light of what has been shown regarding the accelerating action of certain of the phenols on peroxidase oxidations, that laccol might also function as a peroxidase accelerator; and that

[1] Ber. d. chem. Ges., **35**, 1275–1279 (1902).

[2] Report No. 66, 1901, U. S. Dept. of Agriculture. Also Ber. d. chem. Ges., **35**, 2487–2488 (1902).

[3] Bull. Soc. Chim. [3], **11**, 717–721 (1894).

in its oxidation by laccase, as this occurs normally in the sap of the lac tree, it (laccol) could probably effect the oxidation of other less readily oxidizable substances. It would seem likely, therefore, that the peroxidase accelerators or auxiliary oxygen carriers are of considerable significance for biological oxidations. It is therefore my intention to continue these investigations in the hope of being able to throw further light on this particular phase of the subject.

WASHINGTON, D. C.,
March, 1908.

IMPROVEMENT IN CELLS FOR THE MEASUREMENT OF OSMOTIC PRESSURE.[1]

BY H. N. MORSE AND B. MEARS.

Progress has recently been made in the development of the method for the measurement of osmotic pressure which has contributed materially to the accuracy of the results and the convenience of the work. The improvements have been principally along the three following lines: (1) cell construction and management; (2) the calibration, comparison, and verification of manometers; and (3) the maintenance of constant bath temperatures. All of these are matters of first class importance, and it is thought advisable to devote a short paper to each of them. The present paper deals with improvements in cells.

It is now nearly nine years since the work upon osmotic pressure was begun in this laboratory, and probably more than half of all the time given to the subject has been devoted to experimental work in cell construction. Notwithstanding so much effort, we have been unable, until recently, to provide ourselves with an adequate supply of faultless cells. By "faultless" cells, we mean those in which the solutions systematically develop their maximum pressures. Some account[2] has already been given of the earlier difficulties which

[1] The work upon osmotic pressure which is in progress in this laboratory is aided by grants from the Carnegie Institution. The earlier papers upon the subject will be found in THIS JOURNAL, **26**, 80; **28**, 1; **29**, 173; **32**, 93; **34**, 1; **36**, 1 and 39; **37**, 324, 425, and 558; **38**, 175; **39**, 667; **40**, 1 and 194.

[2] THIS JOURNAL, **34**, 1.

led us to abandon the coöperation of the potters and to attempt to work out the problem of cell making in the laboratory. The problem has proved to be a hard one, and at the present time it is only partially solved. It has long been known that to insure success the pores of the cell wall must be excessively small, and that the porosity must be very uniform throughout, also that the cells must stand burning at very high temperatures without becoming entirely nonporous. But we are still unable to say precisely how all these and still other essential qualities may be secured without any large proportion of failures. During the first five years, we produced about a dozen good cells. Recently about one cell in four has proved to be satisfactory. It is not our purpose, however, to discuss in this paper the problem of cell making, because we are still far from having solved it. We wish, rather, to deal with the form of the cell and the methods of closing it for the measurement of pressure.

Figure 1 (reproduced from a former paper[1]) represents the cell which was employed in the earlier work, and which, with certain modifications, is still used to some extent. Its construction and the purpose of the different parts are sufficiently obvious, so that it is necessary only to call attention to its weak points.

The membrane, when once deposited on the cell wall, must not be subjected to any strain except that exerted directly outward by the osmotic pressure; in other words, it must not be disturbed by any of the operations connected with the closing and opening of the cell. To avoid this, the glass tube, B, was rigidly fixed in the cell, A. The lower end of the glass tube was accurately ground to fit the soapstone ring, b. In order to prevent the solution from penetrating the cement above, and at the same time to protect the latter from liquid entering through the exterior wall, the glass tube and the soapstone ring were joined together watertight by means of molten shellac, and the lower end of the tube and ring, and also the whole wall of the cavity, were painted with a solution of rubber. The ring and tube were then pressed

[1] This Journal, **34**, 4.

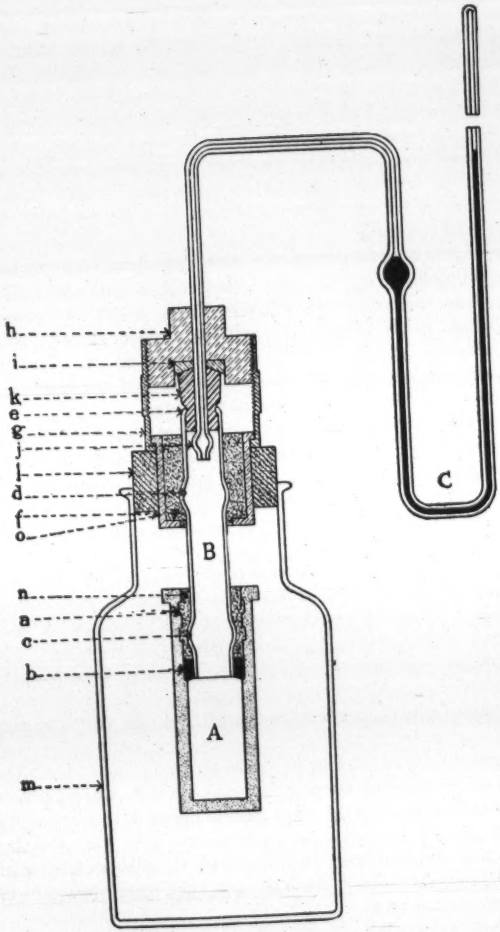


Fig. I.

into place, and the space between the former and the cell wall was filled with the dissolved rubber, after which the rubber lining was hardened by baking at a temperature below the melting point of the shellac. Finally, the space above the ring was filled with the litharge-glycerine cement.

So far as securing a rigid union of glass tube and cell is concerned, the method proved to be perfectly satisfactory; but two difficulties developed, either of which made necessary an occasional reopening and renovation of the cell, and these, as explained in a former paper,[1] are tedious and often unsuccessful operations. After a few months of use, the cell was sure to fail in consequence of a peeling of the rubber from some of the parts protected by it. Again, the membrane becomes, in the course of time, so dense and thick that the passage of water through it is intolerably slow, and it is necessary then to remove it and substitute a thinner one. This cannot be done without opening the cell. The removal of the membrane requires some caution. If it is attempted to dissolve it in strong reagents, like the concentrated mineral acids, the cell wall is sure to be ruined so far as the deposition of a new membrane is concerned. The disadvantages of a compound cell, *i. e.*, one which, like that in the figure, is made up of separate parts which must be securely united, were found to be so great that much time has been spent in devising and testing simpler forms; but before entering upon a description of the new cell, we wish to explain certain other defects of the older one.

It will be seen in the figure that the upper end of the glass tube is considerably contracted; and it is obvious that a great deal of difficulty must be experienced in working the material of the rubber stopper through the small hole at the top into the wider tube below, and also in providing for the escape of solution in order to make room for the entering stopper. The implement which served for both purposes has already been described. It consists of a long piece of round steel with one tapering end, which is bent to a hook form and furrowed out on the underside. The same tool is employed

[1] This Journal, **37**, 325.

in opening the cell, both to admit air and to assist in working the stopper out of the tube. The manipulation in closing and opening such a cell is the most difficult part of the whole work, and it can be entrusted only to the most experienced.

The conduct of the rubber stopper under pressure was responsible for much of the uncertainty pertaining to the earlier measurements, and we propose to explain the difficulties which arose in connection with it and how they were overcome. In the first place, the enlargement on the end of the manometer, j, was driven upwards into the stopper, spreading it and forcing some of the material through the contracted end of the glass tube, e. These movements have frequently been spoken of as "manometer displacement" and "distortion of the stoppers." The effect of them is, of course, to increase the capacity of the cell, and consequently, to dilute the solution. The crowding of the manometer into the stopper was soon overcome by tying the latter very tightly with shoemakers' thread just above the enlargement on the former. But this did not prevent the forcing of some of the material of the stopper out of the glass tube into the space above, that is, the distortion of the stopper continued, though there was no longer any slipping of the manometer. The obvious remedy was to render perfectly rigid the portion of the stopper which is outside of the glass tube, and the simplest device for this purpose was found to be the most effective one. After the stopper had been worked into the cell to the required depth, the whole of the projecting portion, k, was tightly wound with waxed shoemakers' thread, with the result that we were no longer troubled with manometer displacement or distortion of stoppers.

The dilution of the cell contents due to the upward movement of the manometers and of the material of the stoppers was considerable in the first three series of measurements, but in the later ones it was quite negligible, when compared with other sources of dilution for which the rubber stopper is in a large measure responsible. The closing of the cell occupies considerable time, usually from 10 to 15 minutes, and it is clear that during this time the contents of the cell

are under a mechanical pressure which is less than the osmotic pressure, and that water must enter from the wet cell wall over the whole surface of the membrane. The rate of the inflow of water depends upon the difference between the mechanical pressure which is exerted at the stopper and the osmotic pressure of the solution, hence in closing the cell, the operator who holds it and manipulates the manometer endeavors to maintain upon the solution as high and as continuous a mechanical pressure as possible. A more effective remedy, however, for the inflow of water at this period is the practice, employed throughout the later series of measurements, of dipping the cell, before closing, in a solution whose concentration is equal to, or slightly greater than that of the solution within the cell. The dilution of the solutions during the closing period was doubtless considerable in the first three series of measurements. In the later series, it is not believed to have affected seriously the accuracy of the results.

When the cell is to be opened, the hook of the steel implement is inserted between the glass and the rubber in order to relieve the pressure by admitting air to the solution, and the stopper is then slowly withdrawn by twisting it around in the glass tube. Unfortunately, the hook cannot be allowed to remain in place while the stopper is being removed. The form of the tube at the end and the danger of cracking the glass make it necessary to withdraw the instrument after each admission of air, before the work of removing the stopper can be resumed. Hence the contents of the cell are frequently under diminished pressure during the opening period, and the necessary result of this condition is an increased inflow of water from the cell wall. The dilution of the cell contents due to diminished pressure can also be lessened by "dipping," but it cannot be altogether prevented by this means.

The perplexities regarding the proper correction of the results for loss in rotation, which are created by the fact that dilution during the closing period precedes the measurement, while that which occurs during the opening period is subsequent to the measurement of pressure, have already been

discussed at length in former papers, and they need not be reconsidered in a paper in which it is intended to deal with matters of method rather than with results.

The dilution of the cell contents during the opening period was finally overcome by a very simple device, namely, by driving through the stopper, parallel to the manometer tube, a fine hollow steel needle, which was left in place until the manometer and cell had been separated. The solution was thus brought under atmospheric pressure and continued so to the end of the operation. The use of the hollow needle does not alone suffice to prevent all dilution, for it is obvious that with a solution and water on opposite sides of a membrane, the latter must pass into the former as long as the pressure on the solution is less than its osmotic pressure. But when the use of the needle was supplemented by "dipping," it was found that there was in most cases no dilution whatever of the cell contents, in other words, that the solutions, as a rule, suffered no loss in rotation while in the cells.

A serious difficulty with the old form of cell arises from the fact that the time required for closing it is quite long, and that it must be held in the warm hand throughout the operation. The ill consequences of this are twofold; in the first place, our experience has shown us that the good conduct of a membrane depends very much upon the maintenance of a constant temperature from the time of its deposition to the end of the measurement of pressure. On that account, it has been our practice to deposit or repair the membrane at the temperature at which the measurement is to be made, and thereafter to maintain the cell at that temperature until the experiment is finished. Again, if the solution is expanded by holding the cell in the warm hand, and, while it is in that condition, the cell is closed—fixing its capacity—the subsequent fall in temperature in the bath is attended by a dilution of the solution. The custom of wrapping the cell in non-conducting material and of wearing a glove upon the hand which holds it, or of frequently dipping the hand in cold water, is an uncertain remedy for the difficulties which have

been enumerated; the true remedy is a cell which can be more quickly closed.

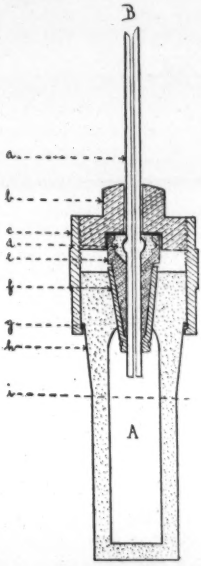


Fig. II.

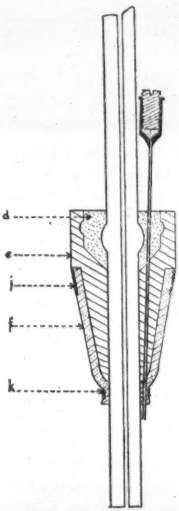


Fig. III.

Fig. II. represents a new form of cell which is entirely free from the objectionable features of the older one. The upper end of the cell is glazed, inside and out, to the line, *i*, or to any other depth, according to the area which the membrane is required to have. In some cases, where it was desired to experiment with very small membranes—as will doubtless be necessary with the electrolytes—the whole cell was covered with the glaze, which was afterwards ground away over and under the spot on which the membrane was to be deposited. Considerable difficulty was experienced in securing a suitable glaze. We first tried a material which is used by the potters

on the better class of tableware. But this was found to "craze" badly when applied to our cells, showing that its expansion coefficient was too small. However, by gradually adding silica and feldspar to the potters' material, we finally obtained a glaze which was continuous and which did not crack when subjected to sudden changes of temperature.

After baking, and before glazing, the cell is placed in the lathe, and the upper end of it is "shaped up" with a carborundum grinder. The conical opening is ground to a true taper, and the outside of the cell is cut down to fit the brass collar, *c*.

The brass cone, *e* (Figs. II. and III.), is firmly attached to the manometer by means of Wood's metal, *d*. The rubber cap, *f*, is slipped over the brass cone, *e*, and securely tied at its two extremities (*j* and *k*, Fig. III.). The tying of the rubber at *k* with twisted shoemakers' thread, and the hard pressing of the rubber above between the conical brass and porcelain surfaces do not alone suffice to prevent a difficulty which was experienced with the old form of cell. The portion of the rubber which is not forced into the cell must be rigidly supported, otherwise there is a slow upward movement of the rubber below, and a condition is created which is quite analogous to the "distortion" of the stopper in the old cell. The manometer cannot "rise" in this case, but the capacity of the cell is increased by the gradual upward movement of that portion of the rubber which is under greatest pressure, and this is followed by a dilution of the solution. For this reason, the upper end of the rubber cap (*j*, Fig. III.) is tightly wound with waxed shoemakers' thread, and it is quite essential to continue the winding so far down that no unsupported rubber is left above the top of the cell, that is, the wound portion must extend somewhat into the neck of the cell.

The closing of the new cell is a very simple operation which may be finished in less than a minute. A fine hollow steel needle is placed between the rubber, *f*, and the wall of the cell to provide for the escape of superfluous solution. The brass nut, *b* (Fig. II.)—which is slotted so that it may be readily separated from the manometer, and also to make room

for the needle—is placed in position and held with a wrench while the brass collar, *c*, is slowly turned with the hand. When the manometer has been made sufficiently firm in its place, the needle is withdrawn, and the turning of the collar is continued until the required amount of "initial" pressure has been produced.

The opening of the cell is equally easy and expeditious. The collar is turned in the reverse direction until the nut can be removed. If the manometer is not wholly released by this operation, the sharp pointed hollow needle is driven in through the rubber, or between it and the neck of the cell, after which, the manometer and cell can be separated without fear of subjecting the solution to diminished pressure.

In order to protect the cell from injury by the turning brass collar, a ring of lead is inserted between them at *g*. A less rigid material, like rubber, leather, or "fiber," cannot be employed for this purpose, because any gradual decrease in the vertical thickness of the ring is attended by a corresponding upward displacement of the manometer.

The practice of "dipping" is also employed in connection with the new cells, notwithstanding the fact that the solutions are never under less than atmospheric pressure. The reason for continuing the custom will be clear when it is recalled that dilution of the solution in the cell must ensue whenever the pressure upon it is less than its potential osmotic pressure.

The procedure just described requires a certain amount of good judgment as to the proper time for removing the needle when a cell is being closed. Moreover, it is sometimes found that the needle is withdrawn with difficulty when the manometer is firmly fixed in its place in the cell. Good judgment is especially necessary in the case of dilute solutions. If the needle is prematurely withdrawn, it will be found that screwing down to maximum permissible "initial" pressure does not suffice to fix the manometer firmly in its place. It is not allowable, as a remedy in such cases, to establish an initial pressure which is greater than the osmotic pressure, because this always produces a concentration of the solution, and, consequently, an abnormally high osmotic pressure. The

reason is quite obvious, and our experience emphasizes the fact that *the mechanical pressure upon the solution must never exceed the maximum osmotic pressure.* We have no hesitation in affirming again that it is a mistake to suppose that osmotic pressure can be measured by increasing the mechanical pressure until it is in equilibrium with the osmotic pressure, or by raising the former above the latter and then waiting for equilibrium to establish itself.

Fig. III. shows a disposition of the needle which is especially advantageous for dilute solutions, and which is not objectionable in the case of concentrated ones. The needle, as indicated in the figure, is permanently fixed in the brass piece, *f*, and provision is made at the top for permitting or stopping, at will, the outflow of solution from the cell. The upper end of the needle is shown on a larger scale in Fig. IV.

Fig. IV.

We have also succeeded, after considerable trouble, in making a cell similar to the one described, which does not require glazing—one in which the upper half is a dense and water-tight porcelain, while the lower half is porous and in every way suited to the deposition of a membrane. The problem to be solved was, of course, so to shade the two different clays through a certain distance in the middle of the cell

that there could not be an abrupt change in expansion coefficient at any point between the porcelain above and the porous part below.

JOHNS HOPKINS UNIVERSITY,
May 29, 1908.

VISCOSITY AND FLUIDITY.

[PRELIMINARY PAPER.]

BY EUGENE C. BINGHAM.

Efforts have been made in earlier papers[1] to show that the fluidities of liquid mixtures are normally a linear function of the composition of the mixture. In investigating pure liquids, it is now found that for unassociated substances, the fluidities are approximately a linear function of the temperature of the substance. This relation may be expressed by the formula

$$t_1 = A\phi + K_1 \qquad (1),$$

where t is the temperature absolute, ϕ the fluidity, and A and K_1 are constants. But associated compounds, the alcohols especially, depart widely from the linear type. At high temperatures their fluidities approximate the linear type, but at low temperatures the fluidity curves do not reach a zero value, but appear to approach the zero value asymptotically, as already predicted by Bousfield and Lowry[2] and by Kohlrausch.[3] To represent the increase in temperature required to give the fluidity ϕ on account of the association of the substance, use is made of the expression

$$t_2 = K_2 - \frac{B}{\phi} \qquad (2),$$

where B and K are constants. Adding together these two equations, we have

$$t = t_1 + t_2 = A\phi - \frac{B}{\phi} + C \qquad (3).$$

[1] *Cf.* THIS JOURNAL, **34**, 481 (1905); Z. physik. Chem., **57**, 193 (1906); Carnegie Institution Publication No. **80**, 107 (1907); THIS JOURNAL, **35**, 195 (1906).

[2] P. Roy. Soc., **71**, 42 (1902).

[3] *Ibid.*, **71**, 338 (1903).

For many purposes it is highly desirable to have an equation with no more than three constants. Thorpe and Rodger, in their classic investigation,[1] limited themselves to equations with but three constants, and they finally adopted Slotte's equation,[2]

$$\eta = \frac{c}{(a+t)^n},$$

as the best equation at their disposal with which to represent viscosity data. Hence, the above equation (3) must be used to compare with Slotte's equation. Too much, however, must not be expected from it, because it neglects to consider the fact that as the number of particles is increased by the breaking down of association, these new particles also contribute to the increase in fluidity in a linear manner. An approximate equation containing four constants (a larger number of constants than this renders the equation unwieldy) is

$$t = A\phi - \frac{B}{\phi + D} + C \qquad (4).$$

The extremely accurate data of Thorpe and Rodger naturally served as a basis for testing the above equations. Eliminating the highly associated classes of substances, acids, alcohols, and water, the results obtained for the 68 remaining substances examined by them are given by equation (3) with an average difference of only 0.08 per cent. Slotte's equation for the same substances, as far as they gave the data, result in an average difference for 58 substances of 0.14 per cent. If the acids, alcohols, and water are included in the test of equation (3), then the average difference for 87 substances rises to 0.17 per cent. The highest average difference is 1.17 per cent in the case of active amyl alcohol. Slotte's equation did not hold at all for many of these, in one case, the equation calculated for one part of the curve giving a difference of over 800 per cent at another part of the curve. It may be remarked here that practically a thousand duplicate observations are at the basis of the above average.

[1] Phil. Trans. [A], **185**, 307 (1894), and **189**, 71 (1897).
[2] Beibl., **16**, 182 (1892).

The following table for hexane will serve to exemplify the above agreement:

ϕ.	Temperature observed.	Temperature calculated.	Per cent difference.	Per cent difference. T. & R.
254.39	273.80	273.80	0.00	—0.03
279.2	282.15	282.44	+0.10	+0.17
296.0	287.75	287.97	+0.07	+0.09
312.3	292.98	293.14	+0.05	+0.07
329.5	298.39	298.43	+0.01	—0.03
345.54	303.23	303.24	+0.00	—0.03
367.3	309.76	309.59	—0.05	—0.11
391.2	316.47	316.28	—0.06	—0.04
404.9	320.42	320.09	—0.10	—0.16
425.3	325.90	325.67	—0.07	—0.09
448.2	331.76	331.77	+0.00	+0.05
466.64	336.59	336.61	+0.01	0.00

Average per cent difference: equation (3), 0.04; Thorpe and Rodger, 0.07. Constants in equation (3): $A = 0.21892$, $B = 9137.8$, $C = 254.03$.

In testing associated substances by means of equation (4) the agreement with the observed values is equal to that with the simpler equation (3) and unassociated substances. Of course, equation (4) may be used in the case of those substances also, wherever the agreement is not already within the limits of experimental error. Active amyl alcohol gives an average difference of 1.17 per cent with equation (3). With equation (4) this becomes only 0.08 per cent.

Duff[1] has recently given equations with four constants which seem to give closer agreement than any heretofore proposed. The equations are somewhat unwieldy and have not been applied to many substances, so an extensive comparison cannot be made. The average difference obtained by Duff between the observed and the calculated values is as follows: for isobutyl alcohol, 0.35 per cent; for dimethylethylcarbinol, 0.24 per cent; and for water, 0.18 per cent. Equation (4) gives for these an average difference of 0.09, 0.08, and 0.01 per cent, respectively. The following table shows the nature of this agreement in the case of water.

[1] Physic. Rev., 4, 404 (1896).

ϕ.	Temperature observed.	Temperature calculated.	Per cent difference.	Per cent difference. T. & R.	Per cent difference. Duff.
56.91	273.37	273.37	0.00	+0.2	+0.3
58.48	274.13	274.17	+0.01	+0.2	+0.3
62.19	276.09	276.02	—0.02	+0.1	—0.2
65.15	277.47	277.47	0.00	0.0	0.0
66.93	278.45	278.33	—0.04	0.0	+0.4
72.62	281.01	281.02	+0.00		
84.67	286.53	286.48	—0.02	+0.2	—0.4
104.71	295.02	294.93	—0.03	—0.4	—0.4
127.23	303.72	303.68	—0.01	—0.2	—0.1
151.06	312.32	312.32	0.00	0.0	0.0
173.61	320.03	320.03	0.00	+0.2	+0.2
199.60	328.53	328.48	—0.02	+0.2	+0.2
226.50	337.01	336.85	—0.05	+0.1	+0.1
255.43	345.54	345.51	—0.01	+0.4	+0.4
283.69	353.75	353.71	—0.01	+0.3	+0.1
315.96	362.90	362.81	—0.02	0.0	0.0
346.02	371.09	371.09	0.00	0.0	0.0
353.35	373.00	373.08	+0.02	0.0	0.0

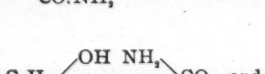
Average per cent difference: equation (4), 0.01; Thorpe and Rodger, 0.15; Duff, 0.18. Constants in equation (4): $A = 0.23275$, $B = 8676.8$, $C = 309.17$, $D = 120$.

RICHMOND COLLEGE, RICHMOND, VA.,
March 19, 1908.

UREIDES AND CYANAMIDES OF THE DIALKYLOXY-ACETIC ACIDS.

BY ERIK CLEMMENSEN AND ARNOLD H. C. HEITMAN.

The ureides of the oxy-fatty acids are represented only by the hydantoins, $C_nH_{2n}\begin{cases}\text{NH.CO}\\ \text{CO.NH}\end{cases}$, and the hydantoic acids, $C_nH_{2n}\begin{cases}\text{NH.COOH}\\ \text{CO.NH}_2\end{cases}$, while the real ureides,



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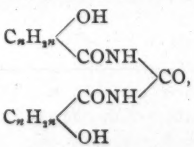
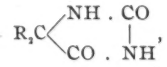


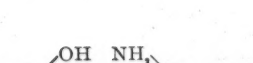
have not been made. By real ureides of the oxy-fatty acids we understand compounds formed by substituting one or more of the hydrogen atoms in ureas with the radicals of the oxy-fatty acids, $C_nH_{2n}\begin{matrix}OH\\ \diagdown\\ CO-\end{matrix}$. Hence these compounds will still contain the characteristic alcoholic hydroxyl groups, and their C_nH_{2n} groups will not be linked directly to the nitrogen atoms as in the hydantoins and the hydantoic acids. We therefore thought it would be of interest to try to prepare them and study their properties.

For several reasons we started with the dialkyloxyacetic acids, and this paper will deal exclusively with these; but we have already begun the work on some of the other oxy-fatty acids, especially the glycollic and the lactic acids, the result of which we intend to publish later.

Errera[1] has prepared and described the *C*-dialkylhydantoins,



whereas the real *C*-dialkyloxyacetylureides,



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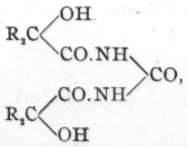
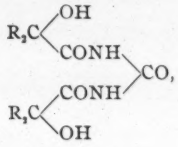


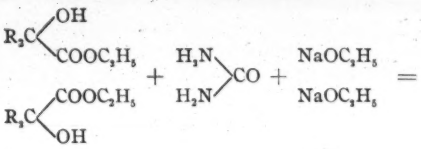
as already mentioned, have not been made before. A. Michael[2] obtained malonylurea by condensing diethyl malonate and urea by means of sodium alcoholate, and E. Fischer and A. Dilthey[3] have made the *C*-dialkylmalonylureides in practically the same way. We, therefore, had every reason to expect a good result by using this method in making the *C*-dialkyloxyacetylureides, and this was entirely confirmed, for by treating

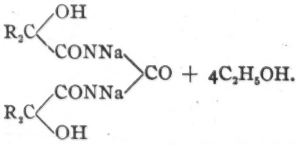
[1] Gazz. chim. ital., **26, I**, 197.
[2] J. prakt. Chem. [2], **35**, 456.
[3] Ann. Chem. (Liebig), **335**, 334.

the esters of the dialkyloxyacetic acids with urea and sodium alcoholate, we obtained ureides of the formula



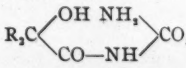
the following reaction taking place:





The reaction takes place at water bath temperature with a highly satisfactory yield.

On the other hand, although we varied the process in many ways, we were not able to obtain the ureides of the type,



corresponding to urea in which only one of the hydrogen atoms has been substituted with the acid radical.

The new ureides are low melting, very stable substances which can be distilled under reduced pressure without decomposition. They act as rather strong dibasic acids.

Michael[1] obtained malonylthiourea and malonylguanidine by using thiourea or guanidine in his process instead of urea,

[1] J. prakt. Chem. [2], **35**, 456.

and the thioureides and guaneides of the dialkylmalonic acids were prepared in a similar way by Fischer and Dilthey.[1] However, when we tried to make the thioureides and guaneides of the dialkyloxyacetic acids by treating the esters and thiourea or guanidine with sodium alcoholate, we came to a very different result, as in both cases we obtained the same dialkyloxyacetylcyanamides, $R_2C\begin{matrix}OH\\ CO.NH.CN\end{matrix}$, compounds which have not hitherto been made.

No doubt, in the presence of the dialkyloxyacetic esters the sodium alcoholate converts the thiourea and the guanidine into sodium cyanamide:

$$C(NH)\begin{matrix}SH\\ NH_2\end{matrix} + NaOC_2H_5 = C\begin{matrix}\equiv N\\ NHNa\end{matrix} + H_2S + C_2H_5OH;$$

$$C(NH)\begin{matrix}NH_2\\ NH_2\end{matrix} + NaOC_2H_5 = C\begin{matrix}\equiv N\\ NHNa\end{matrix} + NH_3 + C_2H_5OH;$$

and the sodium cyanamide formed reacts in turn with the esters, forming the dialkyloxyacetylcyanamides:

$$R_2C\begin{matrix}OH\\ COOC_2H_5\end{matrix} + \begin{matrix}N\\ HNaN\end{matrix}\!\!\equiv\!\!C = R_2C\begin{matrix}OH\\ CO.NNa.CN\end{matrix} + C_2H_5OH.$$

The whole reaction, which takes place on treating a mixture of the dialkyloxyacetic esters and thiourea or guanidine with sodium alcoholate, is therefore as follows:

$$R_2C.OH.COOC_2H_5 + CS(NH_2)_2 + 2C_2H_5ONa = R_2C.OH.CO.NNa.CN + NaSH + 3C_2H_5OH.$$

[1] Ann. Chem. (Liebig), **335**, 334.

We are unable to explain why this reaction takes place here, while the dialkylmalonic esters under the same conditions yield the thioureides and guaneides, but the hydroxyl groups no doubt play an important rôle, and further investigation, which we hope to carry on in the near future, will show whether or not this reaction is general for the oxy-fatty acids. As it seemed to us of considerable interest to ascertain whether the dialkylmalonylcyanamides could possibly be formed in this way, we repeated the experiments of Fischer and Dilthey with several modifications, but in all cases, however, we obtained the thioureides and the guaneides. In this connection we might remark that the low yield of diethylmalonylthiourea which Fischer and Dilthey[1] obtained possibly was due to the high temperature and the large excess of sodium alcoholate they employed, since on boiling an alcoholic solution of one molecule thiourea and one molecule diethylmalonic diethyl ester with two molecules sodium alcoholate, the yield of diethylmalonylthiourea exceeded 80 per cent of the theoretical.

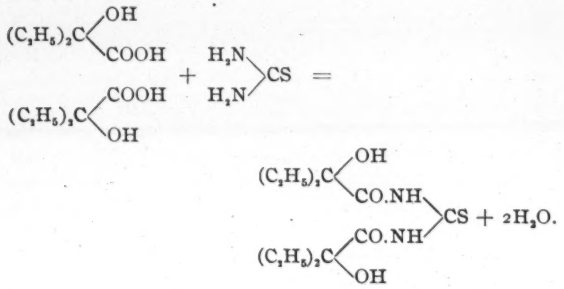
The new cyanamides are high melting, well crystallizing substances, which by boiling with acids or alkalies are quantitatively converted into the *C*-dialkyloxyacetylureides, the following reaction taking place:

$$2R_2C\begin{matrix}OH\\ CO.NH.CN\end{matrix} + 3H_2O = R_2C\begin{matrix}OH\\ CO.NH\end{matrix}\!\!>\!CO + 2NH_3 + CO_2.$$
$$R_2C\begin{matrix}CO.NH\\ OH\end{matrix}$$

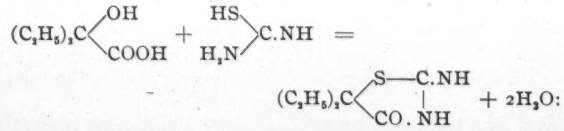
Since, therefore, we were unable to form the *C*-dialkyloxyacetyl thioureides and guaneides in this way, we tried several other methods, letting, for instance, phosphorus haloids and similar condensing agents act on mixtures of the acids and thiourea or guanidine, but without success, probably on account of the great tendency of the oxyacids to form anhydrides in the

[1] Ann. Chem. (Liebig), **335**, 350.

presence of these bodies, and it was by heating a mixture of diethyloxyacetic acid and thiourea to 180° that we first succeeded in obtaining *C*-diethyloxyacetylthiourea, the following reaction taking place:



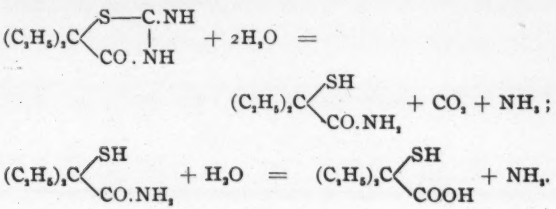
At the same time, however, another reaction takes place, as the alcoholic hydroxyl group takes part in the reaction, causing the formation of another compound hitherto unknown, namely, *C*-diethylthiohydantoin, and this secondary reaction predominates when the temperature is raised to 200° and an excess of thiourea is used. Thiourea must here react as follows:



since the *C*-diethylthiohydantoin formed is converted by boiling with barium hydroxide into *C*-diethylthioglycolamide,

and by prolonged boiling into diethylthioglycollic acid,

two compounds which have not been made before, the following reactions taking place:



Unfortunately we did not have enough of the two substances, which we have designated as diethylthioglycollic acid and diethylthioglycolamide, to make a complete analysis. However, as the sulphur and nitrogen determinations gave results in full accordance with the theory, we do not doubt that these formulas are correct. It is futhermore in accordance with the results of Andreasch,[1] who found that the ordinary thiohydantoin, by boiling with baryta, yielded thioglycollic acid. The intermediate product, thioglycolamide, was, however, not formed in his case. We hope to be able to carry out a closer examination of all these thio compounds and to give the results in a later paper.

EXPERIMENTAL.

C-Diethyloxyacetylurea, $\begin{matrix}(C_2H_5)_2C.OH.CO.NH\\(C_2H_5)_2C.OH.CO.NH\end{matrix}\!\!>\!CO$.—The ethyl diethyloxyacetate we used was made by Fittig's modification[2] of Frankland and Duppa's process,[3] according to which one molecule ethyl oxalate and two molecules ethyl iodide are heated with an excess of granulated, slightly amalgamated zinc and small quantities of zinc ethyl and ether. The addition of zinc ethyl is inconvenient, and in our opinion not at all necessary. Our experience is that the yield depends mostly on the physical condition of the zinc, the spongy,

[1] Ber. d. chem. Ges., **12**, 1385.
[2] Ann. Chem. (Liebig), **200**, 21.
[3] *Ibid.*, **138**, 109.

impure zinc which can be obtained on the market giving the best results. This was treated a short time with dilute sulphuric acid, then washed, dipped a few minutes in a 0.1 per cent solution of mercury chloride,[1] then washed carefully again and dried. By using equal parts of the nonamalgamated zinc (treated with sulphuric acid) and the amalgamated zinc, we obtained over 90 per cent of the theoretical yield. The ester boiled at 173°–175°.

In order to prepare the ureide, 5 grams sodium were dissolved in 100 cc. absolute alcohol, and to the cooled solution were added 32 grams ethyl diethyloxyacetate and 7 grams urea, care being taken to have both the materials and apparatus perfectly dry. The whole was then boiled in a flask with reflux condenser for about 5 hours. The reaction product, containing the sodium salt of the diethyloxyacetylurea, was slightly acidified with dilute hydrochloric acid, the alcohol distilled off, and the ureide, which formed an oily layer on top of the salt solution, separated from this, washed with a little cold water, dried, and then distilled *in vacuo*. A little water and ester first passed over and then the ureide at 175° under 20 mm. pressure. After a second distillation the whole amount of substance boiled constantly at 174°–176° under 20 mm. pressure. The yield was 25 grams, which is about 87 per cent of the theoretical.

The ureide thus obtained is a colorless, oily liquid, having a specific gravity of 1.1107 at 15°. At first we were not successful in our various efforts to crystallize it.

0.1899 gram substance gave 0.3750 gram CO_2 and 0.1363 gram H_2O.

0.1880 gram substance gave 16.5 cc. N at 28° and 751 mm.

	Calculated for $C_{13}H_{24}N_2O_6$.	Found.
C	54.17	53.86
H	8.33	7.98
N	9.72	9.52

The ureide probably still contained small quantities of impurities, and as we thought that these might prevent it from crystallizing, we tried to purify it further. The substance was

[1] Ann. Chem. (Liebig), **200**, 21.

boiled with magnesia, and the filtrate, containing the magnesium salt, precipitated with silver nitrate. The silver salt was collected, washed with cold water, and recrystallized from boiling water, again washed, and dried. The dry salt was dissolved in absolute alcohol, decomposed with hydrogen sulphide, and the filtrate from the silver sulphide freed from alcohol by evaporation. On leaving the remaining colorless oily liquid over sulphuric acid, it solidified to a mass of long, needle shaped, colorless crystals, which begin to soften at 28° and melt at 30°–31°. The substance was again converted into the silver salt, and this treated in the manner described above. The resulting substance had the same melting point, 30°–31°.

0.2575 gram substance gave 0.5106 gram CO_2 and 0.1897 gram H_2O.

0.3012 gram substance gave 27.1 cc. N at 26° and 747 mm.

	Calculated for $C_{12}H_{24}N_2O_5$.	Found.
C	54.17	54.08
H	8.33	8.19
N	9.72	9.81

Instead of the silver salt the mercury salt may be used. When once in possession of a crystal, it is not necessary to use the silver salt to get the ureide in the crystalline form, as the distilled substance readily cystallizes in contact with a crystal already formed.

C-Diethyloxacetylurea can also be obtained by boiling diethyloxyacetylcyanamide (see below) with dilute sulphuric acid (20 per cent), and shaking the ureide out with ether. After evaporation of the ether, a colorless oil remains, which on standing a short time solidifies to a colorless, crystalline mass, melting at 30°–31°.

0.3112 gram substance gave 0.6167 gram CO_2 and 0.2271 gram H_2O.

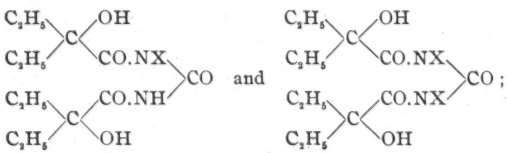
0.2312 gram substance gave 20.9 cc. N at 27° and 753 mm.

	Calculated for $C_{12}H_{24}N_2O_5$.	Found.
C	54.17	54.05
H	8.33	8.11
N	9.72	9.88

One hundred parts of water dissolve about 6.5 parts of the ureide at 20°, and about 14 parts at 100°. It is very easily soluble in alcohol, ether, chloroform, acetone, carbon disulphide, and benzene, only slightly soluble in warm, and nearly insoluble in cold petroleum ether. It dissolves in cold concentrated hydrochloric and sulphuric acid and separates out again unchanged on diluting with water.

By heating the salts in dry form a distillate was obtained which by repeated fractional distillation was separated into diethyl ketone, boiling at 101°–103°, diethylcarbinol, boiling at 116°–117°, and a compound which boiled at 140°–143°, probably triethylcarbinol.

According to its formula diethyloxyacetylurea should form two series of salts,



but only the normal seem to be capable of existence.

The normal alkali salts are prepared by treating the ureide with the equivalent amount of alkali hydroxide, or by boiling it with the equivalent amount of alkali carbonate and evaporating to dryness. They may be purified by dissolving them in ether-alcohol, filtering, evaporating to dryness, and finally washing with ether. They are then obtained as hygroscopic, saline masses very soluble in water and alcohol, readily soluble in ether-alcohol, and insoluble in ether. They dissolve readily in warm acetone, from which they crystallize on cooling, and are precipitated as sticky masses by addition of ether.

We did not succeed in obtaining the acid salts by using half the amount of alkali.

The *calcium salt*, $C_{13}H_{22}N_2O_5Ca$, is best obtained by boiling the ureide with excess of lime, filtering, and evaporating the filtrate to dryness. It is freed from calcium oxide by

dissolving it in alcohol, and forms a hygroscopic, glassy mass very soluble in water and alcohol, insoluble in ether and acetone.

The *magnesium salt* is made in a similar way. White, non-hygroscopic powder, readily soluble in water, less so in alcohol. From its aqueous solution it is thrown down by acetone as a flocculent precipitate. For analysis it was dried at 100°.

0.5360 gram substance gave 0.0695 gram MgO.

	Calculated for $C_{12}H_{28}N_2O_8Mg$.	Found.
Mg	7.85	7.79

When copper chloride is added to an excess of not too dilute solutions of calcium, barium, strontium, or magnesium salts of diethyloxyacetylurea, double salts of the formula $C_{28}H_{44}N_4O_{10}CuR$ are precipitated as light blue bodies, insoluble in water and alcohol. They are quite soluble in excess of copper chloride, but if these solutions are left standing, green precipitates of the normal copper salt are formed, and the same decomposition takes place by boiling the double salts with water. For analysis the magnesium copper salt was dried at 100°.

0.3529 gram substance gave 0.0427 gram Cu_2S and 0.0592 gram $Mg_2P_2O_7$.

	Calculated for $C_{28}H_{44}N_4O_{10}CuMg$.	Found.
Cu	9.64	9.66
Mg	3.69	3.63

The *normal copper salt* is best obtained by adding a solution of the magnesium ureide to an excess of copper sulphate, and boiling the mixture, or by precipitating a solution of alkali ureide with copper sulphate. It forms a bluish green powder.

If copper sulphate is added to a solution of one molecule diethyloxyacetylurea in one molecule sodium hydroxide, a dark blue, sticky mass is precipitated, and the liquid assumes a dark blue color. Care must be taken not to add the copper sulphate in excess. The precipitate is washed with cold water and dissolved in alcohol. After evaporation

of the alcohol an ultramarine blue powder remains. It is only slightly soluble in water, quite readily in alcohol, easily soluble in chloroform with dark blue color, and nearly insoluble in ether. The content of copper corresponds with that of the acid salt, and by boiling with water it is changed to the normal salt described above. For analysis it was washed with ether and dried *in vacuo* over sulphuric acid.

0.5300 gram substance gave 0.0660 gram Cu_2S.

	Calculated for $C_{28}H_{46}N_4O_{10}Cu$.	Found.
Cu	9.98	9.94

The *zinc salt* is obtained by boiling the ureide with excess of zinc oxide, filtering, and concentrating the filtrate. It then separates out as small, glistening needles, nearly insoluble in cold water, slightly soluble in hot, and insoluble in alcohol. It contains two molecules water of crystallization, which it does not lose at 100°; the water, however, is given off at 130°, but at the same time the salt begins to decompose. For analysis it was washed with alcohol and dried at 100°.

0.3200 gram substance gave 0.0665 gram ZnO.

	Calculated for $C_{18}H_{32}N_2O_8Zn.2H_2O$.	Found.
Zn	16.88	16.70

The *silver salt* is precipitated as a white sticky mass by adding silver nitrate to a solution of the magnesium ureide. Recrystallized from hot water it forms shining, monoclinic prisms. On being heated with water it melts and then dissolves. It is quite readily soluble in hot water, less so in cold, and easily soluble in absolute alcohol, chloroform, and acetone, insoluble in ether. Dried in the air and *in vacuo* over sulphuric acid it still contains two molecules water of crystallization, from which it cannot be freed without decomposition. For analysis it was dried *in vacuo* over sulphuric acid.

0.3860 gram substance gave 0.1548 gram Ag.

	Calculated for $C_{18}H_{32}N_2O_8Ag_2.2H_2O$.	Found.
Ag	40.13	40.10

Mercury chloride precipitates the *mercury salt* as a white powder, nearly insoluble in cold and quite readily soluble in boiling water, from which it crystallizes in silky needles. It is readily soluble in alcohol and acetone and is precipitated from these solutions by addition of water; is easily soluble in chloroform, and insoluble in ether. Dried at 100° it retains one molecule water of crystallization.

0.3880 gram substance gave 0.1790 gram HgS.

	Calculated for $C_{18}H_{32}N_2O_5Hg.H_2O$.	Found.
Hg	39.68	39.86

C-Dipropyloxyacetylurea, $\begin{matrix}(C_3H_7)_2C.OH.CO.NH\\(C_3H_7)_2C.OH.CO.NH\end{matrix}\!\!>\!CO$.—This compound was made in the same way as the diethylureide, by dissolving 5 grams sodium in 100 cc. absolute alcohol, adding to the cooled solution 37.6 grams ethyl dipropyloxyacetate and 7 grams urea, and boiling for some hours. The reaction product was slightly acidified with diluted hydrochloric acid, the alcohol distilled off, and the oily layer of dipropyloxyacetylurea separated from the salt solution, washed with water, dried, and distilled *in vacuo*. The main portion passed over at 188° under 25 mm. pressure, and after a second distillation boiled constantly at 186°–188° under 25 mm. pressure. The yield was 26 grams, which is about 76 per cent of the calculated.

0.3502 gram substance gave 0.7584 gram CO_2 and 0.2823 gram H_2O.

0.3077 gram substance gave 23.8 cc. N at 26° and 738 mm.

	Calculated for $C_{17}H_{32}N_2O_5$.	Found.
C	59.30	59.06
H	9.30	8.96
N	8.14	8.33

It forms a colorless, oily liquid, which by standing in the cold slowly solidifies to a white, crystalline mass. In contact with a fragment of the substance already crystallized, it solidifies instantly. On being heated it begins to soften at 35° and melts at 39°.

C-Dipropyloxyacetylurea may also be obtained by boiling dipropyloxyacetylcyanamide (see below) with diluted sulphuric acid (10 per cent), and shaking the ureide out with ether. After evaporation of the ether a colorless oil remains, which, without having been distilled, solidifies to a white crystalline mass on standing a short time in a cool place. Melting point 39°.

0.3122 gram substance gave 0.6778 gram CO_2 and 0.2557 gram H_2O.

0.2132 gram substance gave 16.4 cc. N at 26° and 741 mm.

	Calculated for $C_{17}H_{30}N_2O_5$.	Found.
C	59.30	59.21
H	9.30	9.10
N	8.14	8.32

The ureide is only slightly soluble in cold water, more so in hot, easily soluble in alcohol, ether, acetone, chloroform, and benzene, difficultly soluble in petroleum ether. It acts as a dibasic acid.

The alkali salts are easily soluble in water, whereas the other salts are much less soluble than the corresponding salts of diethyloxyacetylurea.

The *magnesium salt* can be made by boiling the ureide with excess of magnesia, filtering, and evaporating the filtrate to dryness. It forms a white powder, only slightly soluble in cold water, more so in hot, and nearly insoluble in alcohol. For analysis it was washed with alcohol and dried at 100°.

0.5121 gram substance gave 0.0563 gram MgO.

	Calculated for $C_{17}H_{30}N_2O_5Mg$.	Found.
Mg	6.65	6.60

The *mercury salt* is precipitated by adding mercury chloride to a solution of alkali dipropyloxyacetylurea. It is nearly insoluble in cold water and dissolves in a large amount of boiling water, from which it separates out in microscopic needles.

The ethyl dipropyloxyacetate we used was obtained exactly in the same way as the ethyl diethyl ester, and although the

yield was not as good, it still was quite satisfactory. Boiling point, 208°–210°.

C-Dimethyloxyacetylurea or α-Oxyisobutyrylurea, $(CH_3)_2CO.OH.CO.NH$ $(CH_3)_2CO.OH.CO.NH$ >CO, was made by dissolving 4.6 grams sodium in 100 cc. absolute alcohol, adding 26.4 grams ethyl dimethyloxyacetate and 7 grams urea to the cooled solution, and boiling for some hours. The reaction product was acidified, the alcohol distilled off, and the dimethyloxyacetylurea shaken out with ether. After evaporating the ether it remained as an oily liquid, which, on cooling, solidified to a crystalline mass. It is best purified by dissolving it in a large amount of boiling petroleum ether, from which it crystallizes in silky, snow white needles, or, on slow cooling, in glistening needles, often several cm. long. It melts at 74°. The yield was 21 grams, which is about 91 per cent of the theoretical.

0.1471 gram substance gave 0.2511 gram CO_2 and 0.0887 gram H_2O.

0.2002 gram substance gave 22.2 cc. N at 27° and 752 mm.

	Calculated for $C_5H_{10}N_2O_3$.	Found.
C	46.55	46.56
H	6.90	6.70
N	12.07	12.11

C-Dimethyloxyacetylurea can also be made by boiling dimethyloxyacetylcyanamide with diluted sulphuric acid, and shaking the ureide out with ether. After evaporation of the ether, it remains as a colorless crystalline mass, which, when recrystallized from boiling petroleum ether, melts at 74°.

0.1735 gram substance gave 0.2952 gram CO_2 and 0.1049 gram H_2O.

0.2125 gram substance gave 23.8 cc. N at 28° and 752 mm.

	Calculated for $C_5H_{10}N_2O_3$.	Found.
C	46.55	46.40
H	6.90	6.72
N	12.07	12.16

Dimethyloxyacetylurea is readily soluble in both cold and hot water, easily soluble in alcohol, ether, acetone, chloroform, and benzene, only slightly soluble in warm, and nearly insoluble in cold petroleum ether.

The alkali and the alkali earth salts of this ureide are made in the same way as the corresponding salts of the diethyloxyacetylurea and resemble them very much.

The double salts $C_{18}H_{28}N_4O_{10}CuR$ (where R = Ca, Ba, Sr, or Mg) are precipitated in the usual way, as light blue substances, but only from quite strong solutions. The *normal zinc salt*, obtained by boiling the ureide with zinc oxide, filtering, and evaporating the filtrate to dryness, is easily soluble in both cold and hot water. It contains two molecules water of crystallization, which it does not lose at 100°.

For analysis it was washed with alcohol and dried at 100°.

0.2220 gram substance gave 0.0542 gram ZnO.

	Calculated for $C_9H_{14}N_2O_5Zn.2H_2O$.	Found.
Zn	19.73	19.62

The *normal silver salt* can be made by boiling the ureide with silver carbonate, or by precipitating a strong solution of alkali ureide with silver nitrate. Recrystallized from a little warm water, it forms small prisms, soluble in both cold and warm water.

Dried *in vacuo* over sulphuric acid it still contains one molecule water of crystallization.

0.3122 gram substance gave 0.1446 gram Ag.

	Calculated for $C_9H_{14}N_2O_5Ag_2.H_2O$.	Found.
Ag	46.54	46.32

The ethyl dimethyloxyacetate we used was made by heating potassium dimethyloxyacetate with ethyl iodide to 130,[1] and only the portion boiling exactly at 150° was employed. Concerning the preparation of dimethyloxyacetic acid we would like to mention that it has been our experience that most of the methods given in the literature are not well adapted for

[1] Fittig: Ann. Chem. (Liebig), 188, 54.

making it in quantities, as either the yield is very low, or the processes tedious and expensive. The only process which gave us a satisfactory yield was Markownikow's.[1] A mixture of acetone, hydrocyanic acid, and diluted hydrochloric acid is left standing for 3 weeks, then boiled 3 days, the acetone distilled off, and the acid shaken out with ether.

C-Diethyloxyacetylthiourea, $\begin{matrix}(C_2H_5)_2C.OH.CO.NH\\(C_2H_5)_2C.OH.CO.NH\end{matrix}\!\!>\!CS$, was prepared as follows: 26.4 grams diethyloxyacetic acid were mixed with 10 grams thiourea and heated in an oil bath to 180° for several hours. The reaction product, which formed a thick, brownish oil, was washed with cold water, and then dissolved in a sufficient amount of boiling water. On letting the solution stand, diethyloxyacetylthiourea separated out, sometimes as crystals, but most frequently in the form of an oil. The mother liquor contained smaller or larger amounts of diethylthiohydantoin, which could be obtained by concentration of the liquid.

The diethyloxyacetylthiourea was again dissolved in a large amount of boiling water, and the solution allowed to stand, whereupon the substance crystallized out, either as long slender, yellow needles, or as small, rhombic prisms. By repeating the recrystallization, the substance was at last obtained colorless. It melted at 117°–118°, and this melting point was not changed by a further recrystallization. For analysis it was dried at 80°.

0.2173 gram substance gave 0.4066 gram CO_2 and 0.1500 gram H_2O.

0.3028 gram substance gave 25.0 cc. N at 23° and 745 mm.

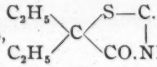
0.1880 gram substance gave 0.1435 gram $BaSO_4$.

	Calculated for $C_{13}H_{24}N_2O_4S$.	Found.
C	51.31	51.03
H	7.89	7.67
N	9.21	9.12
S	10.54	10.50

[1] Ann. Chem. (Liebig), **146**, 339.

The yield was far from satisfactory, however, and in our various efforts to improve it, we did not meet with success.

The substance is only slightly soluble in hot water, insoluble in cold, and easily soluble in most organic solvents. It dissolves readily in alkalies and separates out again on the addition of acids. The substance begins to sublime below 100°. It is a very stable compound, and boiling with acids or alkalies does not seem to affect it.

C-Diethylthiohydantoin,  .—As already mentioned, this compound is formed, together with the thioureide, when a mixture of diethyloxyacetic acid and thiourea is heated to 180°, but a better yield is obtained by raising the temperature to 200° and taking a large excess of thiourea. Accordingly 26.4 grams diethyloxyacetic acid were mixed with 20 grams thiourea and heated to 200° for five hours. The reaction product was washed with cold water, then treated with boiling water, filtered, and the filtrate concentrated. On letting the solution stand, the diethylthiohydantoin crystallized out, frequently mixed with diethyloxyacetylthiourea. These two substances were separated by treatment with ether, in which the thiohydantoin is only slightly soluble, whereas the thioureide is easily soluble. Recrystallized from boiling water it was obtained as colorless crystals, melting constant at 224°. The crystals belong to the regular system and consist mostly of cubes and octahedrons or a combination of these. For analysis the substance was dried at 100°.

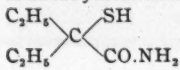
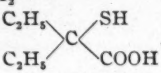
0.1466 gram substance gave 0.2641 gram CO_2 and 0.0955 gram H_2O.

0.1311 gram substance gave 19.7 cc. N at 25° and 745 mm.

0.1040 gram substance gave 0.1420 gram $BaSO_4$.

	Calculated for $C_7H_{12}N_2OS$.	Found.
C	48.82	49.13
H	6.97	7.24
N	16.27	16.42
S	18.63	18.77

The substance is difficultly soluble in cold, readily so in hot water, easily soluble in alcohol and acetone, and very slightly soluble in ether. From chloroform it can be obtained as silky needles. It dissolves in alkalies and is precipitated from these solutions by addition of acids, but redissolves in excess of these.

By boiling with barium hydroxide the *C*-diethylthiohydantoin, as already mentioned, is converted into *C*-diethylthioglycolamide,  , and by continued boiling into diethylthioglycollic acid,  . The operation was carried out as follows: The thiohydantoin was boiled for one hour with an excess of baryta, and the cooled mixture slightly acidified with hydrochloric acid, whereupon an oil and a crystalline substance, both colorless, separated out. They were taken up with ether. After evaporation of the ether the crystals were separated from the oil as well as possible and recrystallized from boiling water. By repeating the recrystallization we finally obtained a substance melting constant at 147°. For analysis it was dried at 100°.

0.1103 gram substance gave 9.9 cc. N at 26° and 743 mm.

0.1078 gram substance gave 0.1721 gram $BaSO_4$.

	Calculated for $C_8H_{15}NOS$	Found.
N	9.52	9.73
S	21.80	21.95

Another portion of thiohydantoin, together with the oil formed in the above experiment, were boiled with baryta for 24 hours, and then made faintly acid with hydrochloric acid. The oil which separated out was shaken out with ether several times, the ether evaporated, and the remaining colorless oil dissolved in just enough boiling water. On cooling, the substance separated out as an oil. On standing *in vacuo* over sulphuric acid, the oil showed signs of crystallization, but as we have already stated, the examination of these two sub-

stances must be regarded as only preliminary on account of lack of material.

The substance did not contain nitrogen, which shows that the diethylthioglycolamide was completely converted into the acid.

0.1231 gram substance gave 0.1909 gram $BaSO_4$.

	Calculated for $C_6H_{12}O_2S$.	Found.
S	21.65	21.32

Dialkyloxyacetylcyanamides.

As already mentioned the dialkyloxyacetylcyanamides were obtained by a process which we expected would yield the thioureides and guaneides, namely, by heating a mixture of the esters of the dialkyloxyacetic acids and thiourea or guanidine with an alcoholic solution of sodium alcoholate. Instead of free guanidine, we employed guanidine sulphocyanide, as this is so easily obtained pure and dry.

Diethyloxyacetylcyanamide, $\begin{matrix} C_2H_5 \\ C_2H_5 \end{matrix}\!\!>\!C\!<\!\begin{matrix} OH \\ CO.NH.CN \end{matrix}$, was made as follows: 4.6 grams sodium were dissolved in 100 cc. absolute alcohol, and to the cooled solution were added 16 grams ethyl diethyloxyacetate and 8 grams thiourea. The mixture was boiled for several hours in a flask with reflux condenser, and then cooled. The reaction product was very carefully neutralized with diluted acetic acid, or better, the acid was added in slight excess, and then neutralized with a solution of sodium carbonate. Upon adding the acid a strong evolution of hydrogen sulphide took place, caused by the action of the acid on the sodium sulphhydrate formed during the reaction. After evaporation of the alcohol on the steam bath, the cyanamide separated out in shining plates, which, after being washed with ether, were recrystallized from boiling water. It then melted constant at 235°. The yield was 13 grams, which is about 83 per cent of the theoretical.

By using 12 grams guanidine sulphocyanide instead of the thiourea, but otherwise conducting the operation in exactly

the same way, an identical substance was obtained, but the yield was lower, namely, 11 grams, which is about 71 per cent of the theoretical. For analysis the substance was dried at 100°.

0.2037 gram substance gave 0.4014 gram CO_2 and 0.1441 gram H_2O.

0.1846 gram substance gave 30.9 cc. N at 29° and 747 mm.

	Calculated for $C_7H_{12}O_2N_2$.	Found.
C	53.85	53.74
H	7.69	7.86
N	17.95	17.94

Crystallized from water it forms colorless, glistening, monoclinic plates. It is only slightly soluble in cold, more so in hot water, and readily soluble in alcohol. It is very sparingly soluble in ether and chloroform, slightly more so in acetone, and practically insoluble in petroleum ether. It is easily soluble in cold diluted alkalies and is precipitated from these solutions by addition of acids, but redissolves in excess of these. On being heated carefully in a test tube, it melts, then volatilizes without decomposition, and condenses in the upper part of the test tube in the form of small crystals.

On boiling with moderately strong acids or alkalies it is quantitatively transformed into diethyloxyacetylurea, the following reaction taking place:

$$2\ \begin{matrix}(C_2H_5)_2\\ \\ (C_2H_5)_2\end{matrix}\!\!>\!C\!<\!\begin{matrix}OH\\ \\ CO.NH.CN\end{matrix} + 3H_2O = \begin{matrix}(C_2H_5)_2C.OH.CO.NH\\ \\ (C_2H_5)_2C.OH.CO.NH\end{matrix}\!\!>\!CO + 2NH_3 + CO_2.$$

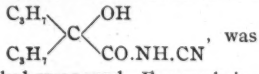
One gram substance was boiled for one hour with 50 cc. sulphuric acid (10 per cent) in a flask with reflux condenser. After cooling, the contents of the flask were carefully neutralized with sodium hydroxide, an excess of magnesia added, the ammonia distilled off, taken up in 0.5 N sulphuric acid, and the

excess of acid titrated with 0.5 N sodium hydroxide: 12.73 cc. acid were used.

The solution remaining in the flask was concentrated, acidified, shaken out with ether several times, the ether evaporated, and the diethyloxyacetylurea remaining dried *in vacuo* over sulphuric acid.

	Calculated for 1 gram diethyloxyacetylcyanamide.	Found.
NH_3	0.1090	0.1082
Diethyloxyacetylurea	0.9231	0.9105

This shows that the yields of ammonia and ureide are respectively 99.3 per cent and 98.6 per cent of the theoretical.

Dipropyloxyacetylcyanamide,  , was made in the same way as the diethyl compound. Four and six-tenths grams sodium were dissolved in 100 cc. absolute alcohol, and to the cooled solution were added 18.8 grams ethyl dipropyloxyacetate and 8 grams thiourea. The whole was boiled for several hours, then cooled, slightly acidified with diluted acetic acid, and the excess of acid carefully neutralized with sodium carbonate. After evaporation of the alcohol, the dipropyloxyacetylcyanamide crystallized out. It was washed with ether. Recrystallized from boiling water, it forms colorless, monoclinic needles, melting constant at 184°. The yield was 15 grams, which is about 82 per cent of the theoretical. By using 12 grams guanidine sulphocyanide instead of thiourea, the same substance was obtained, but the yield was lower. For analysis it was dried at 100°.

0.1831 gram substance gave 0.3939 gram CO_2 and 0.1431 gram H_2O.

0.1781 gram substance gave 24.8 cc. N at 27° and 758 mm.

	Calculated for $C_8H_{16}O_2N_2$.	Found.
C	58.70	58.67
H	8.70	8.68
N	15.22	15.33

Dipropyloxyacetylcyanamide is only sparingly soluble in cold water, more so in hot water, and easily soluble in alcohol.

In ether it dissolves but very slightly, more so in chloroform, and quite readily in acetone. Like the diethyl compound it dissolves easily in diluted alkalies, is precipitated from these solutions by acids, but redissolves in excess of these. On boiling with moderately strong acids or alkalies, it is quantitatively converted into dipropyloxyacetylurea.

Carefully heated above its melting point it volatilizes without decomposition and condenses in the form of small, silky needles.

Dimethyloxyacetylcyanamide or α-Oxyisobutyrylcyanamide, $CH_3(CH_3)C(OH)CO.NH.CN$.—Four and six-tenths grams sodium were dissolved in 100 cc. absolute alcohol, and to the cooled solution were added 13.2 grams ethyl dimethyloxyacetate and 8 grams thiourea. The process was then carried on as usual, and the neutralized solution evaporated to a small volume, whereupon the dimethyloxyacetylcyanamide crystallized on cooling. It was washed with ether and dissolved in just sufficient boiling water, from which it crystallized in tiny, colorless, monoclinic needles, melting at 248°–250°. The yield was 9.5 grams, which is about 74 per cent of the theoretical. For analysis the substance was dried at 100°.

0.1507 gram substance gave 0.2586 gram CO_2 and 0.0857 gram H_2O.

0.2410 gram substance gave 48.2 cc. N at 27° and 750 mm.

	Calculated for $C_5H_8N_2O_2$.	Found.
C	46.88	46.80
H	6.25	6.32
N	21.88	21.77

The dimethyloxyacetylcyanamide is quite readily soluble in cold water, easily so in hot water and alcohol, and practically insoluble in ether, acetone, and petroleum ether. Heated carefully above its melting point it volatilizes and condenses again as small crystals. On boiling with moderately strong acids or alkalies, it is quantitatively converted into *C*-dimethyloxyacetylurea.

From the Laboratories of
Parke, Davis & Co.
May, 1908.

WATER OF CRYSTALLIZATION AS AFFECTED BY LIGHT (I).

By Ralph H. McKee and Elvin J. Berkheiser.

The study of this subject was occasioned by the fact noticed by one of us that of two portions of a preparation of *p*-amino-benzenesulphonic acid, commonly called sulphanilic acid, one portion which had been kept on a shelf and exposed to strong light had apparently lost much more of its water of crystallization than the other portion of the same preparation which had been kept in the dark.

An investigation of the literature failed to disclose any reference to the fact that light had been observed to have an effect on water of crystallization. The references to sulphanilic acid brought out the related facts, first, that the acid cannot be kept in its ordinary crystalline form but gradually "weathers" owing to a loss of water of crystallization and, second, that it can be obtained in a crystalline form free from water when fuming hydrochloric acid is used as the solvent.[1]

Some of the sulphanilic acid used was a "weathered" sample of König's make which had been on our shelves in a clear glass bottle for nearly ten years and had lost all but a trace of its water. The major portion was prepared by heating aniline with concentrated sulphuric acid according to Schmitt's method.[2] Titration of weighed amounts with tenth normal barium hydroxide and phenolphthalein gave excellent agreement with the values calculated from the formula

$$NH_2.C_6H_4.SO_3H.2H_2O.$$

Samples of this hydrated acid were placed in two tubes, A and B. Tube B was like tube A except that it was wrapped in black lightproof paper. These tubes were loosely stoppered with absorbent cotton and exposed side by side to direct sunlight for ten winter days. Examination of the material in each tube by titration of weighed portions with standard barium hydroxide, and by heating at 100° to constant weight,

[1] Laar: Ber. d. chem. Ges., **14**, 1933 (1881).
[2] Ann. Chem. (Liebig), **120**, 132 (1861).

showed that the sample exposed to light had lost more than 98 per cent of its water of crystallization, while the sample wrapped in lightproof paper had lost but 35 per cent.

The temperatures of the samples were determined by two thermometers graduated to tenths of a degree and capable of easy estimation of the hundredths of a degree. A comparison of the two showed that they agreed within the error of reading (0°.01). They were inserted into the tubes until the bulbs were entirely surrounded by the crystals and, as in all the experiments, the tubes were closed with absorbent cotton. For complete certainty, after the temperatures had been once read, the thermometers were interchanged and temperatures read again. Determinations of the temperatures by this method showed that in direct sunlight the thermometer in the tube wrapped in black paper read 30°.42 while that in the unwrapped tube was 2°.86 lower. In diffused light the temperature of the crystals in the wrapped tube was 20°.46 and in the unwrapped tube 1°.36 lower. In other words, the material at the lower temperature, but in the light, lost water faster than the one at the higher temperature, but in the dark.

The possibility that light had produced some change other than simply that of increasing the speed with which the water was given up by the crystals, such as a transformation into the ortho or meta acid, was considered and tested in the following ways:

1. Parallel determinations of the solubility in water of samples which had lost their water of crystallization by heat, and by light, gave results which agreed and were much below what would be expected for the ortho[1] and meta[2] acids. It may be added that the solubility of the anhydrous acid is about 3.5 per cent less than that of the hydrated form, determined simultaneously.[3]

2. Some of the acid which had been exposed to the light was dissolved in dilute sodium hydroxide and precipitated by adding

[1] Thomas: Ann. Chem. (Liebig), **186**, 128 (1877); and Bahlmann: *Ibid.*, **186**, 309 (1877).

[2] Berndsen: *Ibid.*, **177**, 83 (1875).

[3] For the solubilities of the hydrated form of the *p*-aminobenzenesulphonic acid compare Dolinski: Ber. d. chem. Ges., **38**, 1836 (1905).

concentrated hydrochloric acid. After drying at room temperature titration of weighed portions with standard alkali showed that by this recrystallization it had recovered its two molecules of water. The *o*-aminobenzenesulphonic acid crystallizes with 0.5 molecule of water of crystallization and the meta ordinarily water-free, though if a low temperature is used and special care taken it may be obtained with 1.5 molecules of water.

3. When some of the acid which had been exposed to light was recrystallized from warm water and care taken to have the solution so dilute that the separation of crystals did not occur until the temperature had fallen to 40° the crystals obtained had again their two molecules of water.

It was also shown that when sulphanilic acid which either contains its full amount of water or has lost its water by exposure to light was crystallized from water above 80° the crystals obtained were almost entirely free from water.

The fact that light affects water of crystallization is additional evidence to that accumulating in other quarters that such water is in real chemical combination and that we should consider the compound $NH_2.C_6H_4.SO_3H.2H_2O$, for example, as a single molecule instead of one molecule of aminobenzenesulphonic acid and two molecules of water. From this point of view the term "hydrate" to designate a substance with water of crystallization has much in its favor, for in the short term there is little suggestion of the existence in the crystalline substance of water molecules as such.[1]

LAKE FOREST COLLEGE,
LAKE FOREST, ILL.

THE BASICITY OF ACIDS AS DETERMINED BY THEIR CONDUCTIVITIES.

BY M. R. SCHMIDT.

In his work on organic acids,[2] D. Berthelot showed that when an acid is treated with increasing amounts of alkali, its

[1] The investigation is being continued with other substances. The effect of light of various colors is also being studied.—RALPH H. MCKEE.

[2] Ann. Chim. Phys. [6], **23**, and **24** (1891).

conductivity decreases to a minimum, which is reached when sufficient base has been added to neutralize the acid. Further addition of alkali causes the conductivity to increase. This is what would be expected. The swiftly moving hydrogen ions are removed from the sphere of conductivity, owing to the formation of water, and the smaller velocity of the metal ions causes the decrease in conductivity. When, however, an excess of base is added, the presence of a greater number of metal and, especially of hydroxyl ions, causes the conductivity to increase again. This is simply another expression of the fact that the conductivity of a salt is, generally speaking, less than that of its component acid or base.

If this method is applied to polybasic acids, a minimum is found when enough alkali has been added to form the primary salt. Then the conductivity rises and continues to rise even after the secondary salt has been formed, but with a different rate of increase. If the conductivities are plotted as ordinates and the amounts of alkali added as abscissas, a sharp minimum is found at the point corresponding to the primary salt when the dilution is great. The curve then rises to the point denoting the secondary salt, but from here to the point corresponding to the formation of the tertiary salt, a *change of slope* is noticed, and so on. Berthelot[1] employed this method of partial neutralization in the study of phosphoric acid, and came to the conclusion that phosphoric acid is not a true tribasic acid, but a monobasic acid "of complex function," in which the first hydrogen to be replaced by metals resembles the characteristic ion of strong acids, the second the slightly dissociated hydrogen of weak acids, and the third the hydrogen of the hydroxyl group in phenols, etc.

Miolati and Mascetti,[2] using a modification of Berthelot's method, studied the effect of alkalies on the conductivities of solutions of sulphurous, selenous, selenic, telluric, arsenious, arsenic, phosphorus, boric, chromic, iodic and periodic acids. In every case a minimum conductivity was found when suffi-

[1] Ann. Chim. Phys. [6], **38**, 1 (1893).
[2] Gazz. Chim. Ital. **31** [1], 93 (1901).

cient alkali had been added to neutralize one hydrogen, and a marked change in slope was noticed in the curves when the second hydrogen was replaced by metals.

In a recent article,[1] Pellini and Pegararo have shown that the same method, applied to hydrofluoric acid, leads to the interesting conclusion that this substance, in solution, behaves as a dibasic acid. Evidence on this point from other sources is conflicting. On the one hand, the existence of such well-known salts as KHF_2 and $RbHF_2$ seems to lend support to the view that hydrofluoric acid is dibasic. Peratoner and Paterno[2] made some cryoscopic measurements which they interpreted as furnishing evidence for the dibasic character of hydrofluoric acid. They obtained a molecular lowering of the freezing point of about 2°.0, as against 3°.6 for hydrochloric acid; and concluded that hydrofluoric acid should have, in aqueous solution, the formula H_2F_2. But, in the light of the theory of electrolytic dissociation, as Pellini and Pegararo point out, the results show rather that hydrofluoric acid exists in solution as the single molecules, which are only slightly dissociated. Moreover, the application to sodium fluoride and potassium fluoride of Ostwald's rule for determining the basicity of an acid, gives results which indicate that we are dealing with salts of a monobasic acid:

	$V.$	$\mu_{25°}.$	
NaF	1024	104.0	$\Delta = 11.0$
	32	93.0	
KF	1024	126.1	$\Delta = 11.4$
	32	114.7	

If sodium fluoride and potassium fluoride were salts of a dibasic acid, the value of Δ should be about 20.

In its conductivity, as compared with the other halogen acids, hydrofluoric acid shows a further peculiarity. The following figures, taken from Ostwald, indicate, as Pellini and Pegararo state, a wide variation from the behavior of the

[1] Gazz. Chim. Ital. **38** [1], 1 (1908); summarized in Z. Elek. Chem. **13**, 621 (1907).
[2] Rend. Acc. Linc. [4], **6**, 306 (1890).

ordinary halogen acids, on the one hand, and from that of the dibasic acids on the other.

V.	HF. $\mu_{25°}$.	HCl. $\mu_{25°}$.	HBr. $\mu_{25°}$.	HI. $\mu_{25°}$.
4	29.6	366	377	376
32	59.5	393	398	397
1024	224.0	...	405	404

Further illustration of the abnormal character of hydrofluoric acid is shown in the solubility of some of its salts. Silver fluoride, for instance, is easily soluble in water, while silver chloride, bromide and iodide are insoluble. Again, calcium chloride, bromide and iodide are deliquescent, while calcium fluoride is insoluble.

Pellini and Pegararo undertook to settle the question of the basicity of hydrofluoric acid by means of the method of neutralization here described. They worked at 25° and at a dilution of $V = 173$. Reckoning hydrofluoric acid as a monobasic acid, the minimum of conductivity was found when an amount of alkali had been added equivalent to one-half molecule of hydrofluoric acid. This result is in accord with all work previously done on acids known to be dibasic, such as sulphuric, chromic, telluric, etc. Moreover, there is a sudden change in the slope of the conductivity curve when complete neutralization is effected, as would be expected with a dibasic acid. The application of this same general method by Düllberg[1] to a number of acids, including dilute solutions of phosphoric acid, should also be mentioned.

Berthelot's work on phosphoric acid was carried out with solutions of moderate dilution. Work was taken up in this laboratory in order to ascertain the *behavior of phosphoric acid in fairly concentrated solutions*. For this purpose solutions were made up to contain 1, 0.5, 0.2, 0.1 gram molecules (98.03 grams) phosphoric acid in 1000 cc. The N solution was standardized against caustic potash and then checked by gravimetric determinations. The error in the strength of the solutions was probably ±0.3 per cent. In preparing

[1] Z. physik. Chem., **45**, 129 (1903).

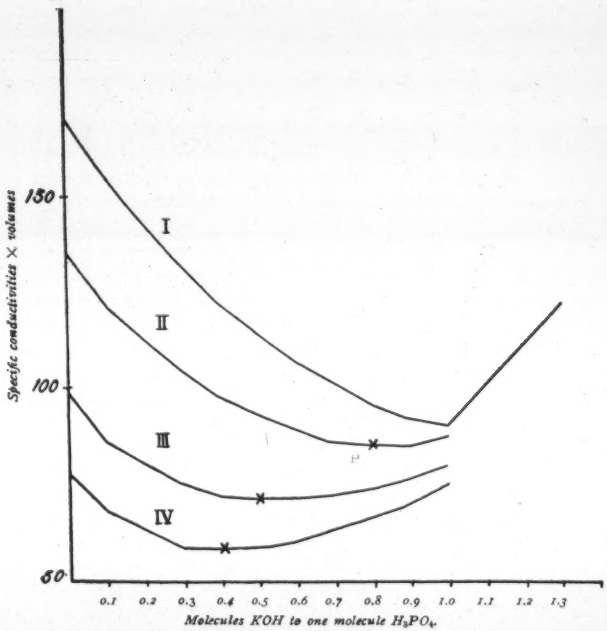
the solutions for conductivity measurements, a plan differing slightly from that adopted by Berthelot, and Miolati and Mascetti was employed. Ten cc. of a phosphoric acid solution were taken, and to this was added one, two, three, etc., cc. caustic potash of the same molecular concentration. The volume of the whole was then brought to 50 cc. at 20°. Thus the solutions of the partially nuetralized phosphoric acid were 0.2, 0.1, 0.04 or 0.02 N with respect to PO_4. Consequently, the conductivities obtained are strictly comparable with other conductivity values, which is not the case with the results of the investigations above named. The values given in the table are equal to $s \times V$, where s is the specific conductivity of the solution; and, of course, in two cases, where the solutions contain only phosphoric acid or only primary potassium phosphate, $s \times V$ becomes equal to μ_v.

The standard of conductivity was that of a 0.02 N solution of potassium chloride at 25°, $\mu_v = 129.7$. All readings were made at 25°, and the conductivities are corrected for the water used.

An attempt was made to obtain measurements for a N solution of phosphoric acid, but, owing to the very small resistances that had to be used, accurate results were not possible.

cc. KOH added.	0.2 N H_3PO_4. $s \times V$.	0.1 N H_3PO_4. $s \times V$.	0.04 N H_3PO_4. $s \times V$.	0.02 N H_3PO_4. $s \times V$.
0	77.8	97.6	134.4	168.8
1	68.0	85.8	121.5	154.8
2	(57.2)	80.1	109.3	142.6
3	58.6	75.3	104.6	131.8
4	58.4	72.2	97.5	121.5
5	59.1	71.4	93.0	114.4
6	60.6	71.5	89.5	106.9
7	63.6	72.2	86.0	101.4
8	67.0	74.3	85.6	95.9
9	70.3	76.9	85.5	92.2
10	75.2	80.1	87.6	90.9
11	....	....	.....	101.9
12	....	....	.....	112.2
13	....	....	.....	122.8

The results are plotted in the accompanying figure.



It will be noticed that only in one case—in the 0.02 N solutions curve I.—does the minimum fall at the point corresponding to the complete neutralization of one hydrogen. In the 0.2 N solutions (curve IV.) the minimum occurs when only about 40 per cent of the amount of the caustic potash required for one-third neutralization has been added. In the more dilute solutions, the minimum shifts continually towards the point corresponding to the salt, KH_2PO_4, reaches it in the 0.02 N curve (curve I.) and then remains stationary, as a trial with 0.01 N phosphoric acid proved.

The cause of this phenomenon has been suggested by Dr.

B. B. Turner. As more and more caustic potash is added, the $\overset{+}{H}$ ions of the phosphoric acid are replaced by $\overset{+}{K}$ ions, which move more slowly, and as a result the conductivity of the mixture decreases. But in a concentrated solution, phosphoric acid is far from completely dissociated. Hence, when a comparatively small amount of base has been added, practically all of the hydrogen ions present are neutralized but more immediately dissociate, and in sufficient quantity to overbalance the decrease in conductivity caused by the substitution of the $\overset{+}{K}$ ions for the $\overset{+}{H}$ ions. Therefore, the conductivity rises again, and a minimum appears before enough alkali has been added to form the primary salt. Furthermore, as the dilution of the phosphoric acid increases, the number of hydrogen ions present becomes greater, and continually increasing amounts of base are necessary to neutralize them. Consequently, a continually decreasing amount of hydrogen remains to dissociate from the H_3PO_4 molecule, and thus the minimum is found at points which continually approach the point corresponding to the salt KH_2PO_4. After a dilution of 0.02 N is reached, the phosphoric acid is dissociated according to the formulation

$$H_3PO_4 \rightleftarrows \overset{+}{H} + \overset{-}{H_2PO_4}$$

to such an extent that the increase in conductivity due to the dissociation of the hydrogen, after the $\overset{+}{H}$ ions already present have been neutralized, is not great enough to make up for the decrease in conductivity, and therefore the curve does not rise again until after the complete neutralization of the first hydrogen.

A method is here indicated by which the conductivity of any of the mixtures may be calculated. On the assumption that in the solutions worked with the second and third hydrogen ions of the acid are formed to only a negligible extent, we have, at the point of equilibrium, the following conditions: The primary potassium salt present in any mixture tends to drive back the dissociation of the phosphoric

acid, and the latter, in turn, tends to suppress the ionization of the salt KH_2PO_4. Hence, if no hydrolysis takes place, we may represent equilibrium by the formula

$$\overset{+}{H} + KH_2PO_4 \rightleftarrows \overset{+}{K} + H_3PO_4.$$

Let the total concentration of phosphoric acid, *i. e.*, of all ions or molecules containing the PO_4 group be represented by a; the total concentration of potassium by b; the undissociated phosphoric acid by x; and the undissociated primary potassium salt by y.

Now, the PO_4 group is present as

$$[H_3PO_4] + [\overset{-}{H_2PO_4}] + [KH_2PO_4],$$

or

$$a = x + [\overset{-}{H_2PO_4}] + y,$$

and

$$[\overset{-}{H_2PO_4}] = a - x - y.$$

Also,

$$a = x + b + [\overset{+}{H}],$$

and

$$[\overset{+}{H}] = a - x - b,$$

and

$$[\overset{+}{K}] = b - y.$$

Hence, if the mass law holds, we have

$$\frac{(a-b-x)(a-x-y)}{x} = K$$

and

$$\frac{(b-y)(a-x-y)}{y} = K'.$$

K and K′ are known, being the ionization constants of phosphoric acid and of primary potassium phosphate, respectively; also, a and b are known for any given mixture. From these values x and y can be calculated, and, since the relative velocities of the ions are known, we can calculate the

conductivity of each mixture of phosphoric acid and potassium phosphate.

In conclusion, I would express my thanks to Professor H. C. Jones, who suggested this investigation, and under whose guidance it was carried out.

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JOHNS HOPKINS UNIVERSITY,
BALTIMORE, April, 1908.

REVIEWS.

MODERN PIGMENTS AND THEIR VEHICLES, THEIR PROPERTIES AND USES CONSIDERED MAINLY FROM THE PRACTICAL SIDE, AND HOW TO MAKE TINTS FROM THEM. By FREDERICK MAIRE, ex-Editor of "Painting and Decorating," author of the "Modern Wood Finisher," the "Modern Grainer." New York: John Wiley & Sons; London: Chapman & Hall, Ltd. 1908. Price, $2.

This book is written by a practical painter, who shows a very thorough knowledge of the materials of his craft. The subject matter includes a detailed description of all pigments used by painters and decorators, with method of manufacture or preparation, physical characteristics, uses and method of application of each. The pigments are divided in groups, based on color, with clear concise statements of the color, tone, tinting power, and general character of each. The chapters on "Compounding of Pigments" and "Test of Principal Tints and Pigments Required to Make Them" will be of much value to the practical painter, and will aid the chemist in the analysis of mixed paints. The table of synonyms is very complete and will be useful until some uniform system of nomenclature is adopted by the paint trade. The chapters on vehicles are rather unsatisfactory and incomplete. No mention is made of China-wood oil or wood turpentine. The chemistry of this subject is weak, as, in fact is the case all through the book. It is unfortunate that misstatements regarding formulae of white lead, red lead, the chemistry of the fixed oils, and theory of drying were not more carefully edited before the book was published. Altogether the volume will be of value to the progressive practical painter, but will be of but limited assistance to the chemist. S. S. VOORHEES.

DETECTION OF COMMON FOOD ADULTERANTS. By EDWIN M. BRUCE, Instructor in Chemistry, Indiana State Normal School. New York: The D. Van Nostrand Company. 1907. pp. 184. Price, $1.25, net.

The book is a compilation of qualitative tests for the detection of food adulterants. It is of value in interesting stu-

dents in the subject, but it is difficult to see what class of people can advantageously use a book, many of whose methods require so much care, experience, and skill and which yet is practically free from quantitative methods. Mohler's method for the determination of benzoic acid on page 10, for instance, requires more experience in its manipulation than is to be expected of one who is not proficient in quantitative as well as qualitative analysis. After the method is described comforting information is given that "If this test is not carefully performed, it is worthless, as other substances give similar results." This is followed by a paragraph directing the concentration of the alkaline solution and precipitation of the benzoic acid by acidifying. The same reaction is also given by other substances, but the author does not state that fact.

On page 8 the statement is made that one may test for nitrates by identifying the metal by a qualitative test for potassium.

The statement of the author on page 16 that alum may be added by millers to cover up bad flour is probably not true at the present time; that stannous chloride and potassium carbonate are added to ginger cake to influence color is also probably erroneous.

The book includes the old phenol and sulphuric acid test for formaldehyde which was used before the modern methods were known, but which has long since been discarded.

The statement that gelatin may be added to increase the weight or thicken the more voluble adulterants of honey is also open to question.

On pages 60 and 63, the author says that lard from hogs fed on any of the various cottonseed products may give a faint reaction by Halphen's test. This would lead the unitiated to suppose that a strong reaction would not result from feeding hogs on cottonseed meal which repeated experiments have shown to be untrue.

W. D. BIGELOW.

STOICHIOMETRY. By SIDNEY YOUNG, D.Sc., F.R.S., Professor of Chemistry in the University of Dublin. With Eighty-eight Figures in the Text. Together with an Introduction to the Study of Physical Chemistry by SIR WILLIAM RAMSAY, K.C.B., F.R.S. 39 Paternoster Row, London; New York; Bombay and Calcutta: Longmans, Green and Co. 1908. pp. 381. Price, $2.

The contents of this admirable volume will show its scope: Fundamental Laws of Chemical Combination, General Properties of Gases, Determination of Atomic Weights, The Periodic Law, The Dissociation of Gases and Vapors, The Prop-

erties of Liquids, The Kinetic Theory of Gases, Properties of Solids, Mixtures, Solubility and Miscibility, Properties of Dilute Solutions, Dissolution and Vaporization, and the Determination of Molecular Weights.

The Introduction to the Study of Physical Chemistry published by Ramsay in Findlay's volume on the Phase Rule is repeated here, with a few additional pages at the end.

The book is very well written, as we would expect from the fact that it comes from the pen of Sidney Young, and is well worthy of the series of which it forms a part. There are, however, a few matters which we could wish were otherwise. On the first page of the text we find a paragraph on "The Conservation of Matter." This would be pretty bad, if the text did not explain so carefully and clearly what was meant. There is, of course, no such law known to man. We have absolutely no means of measuring matter, and even if it exists, do not know whether it is conserved in chemical reaction. What is meant is, of course, the law of the conservation of mass. The discussion of valency (pp. 100 to 104) is unsatisfactory. The subject is well illustrated and this is about all. That valency is a purely electrical phenomenon, governed by Faraday's law, which is its physical basis, is not made clear. Indeed, there are few subjects in any branch of science so unsatisfactorily discussed in general as chemical valence, and this is due primarily to a lack of any definite basis on which to begin the discussion. If we place valence upon a perfectly definite rigid physical basis—Faraday's Law—where it belongs, the discussion of this subject in a scientific manner would at least be made possible.

Another defect in the book is the clumsy appearance of the curve-sheets. The use of the double-lined ordinates, and the very heavy type inside and outside of the curve sheet proper, does not give a neat appearance.

These are, however, minor defects which can easily be corrected in a new edition. H. C. J.

THERMOCHEMISTRY. By JULIUS THOMSEN, Emeritus Professor of Chemistry in the University of Copenhagen. Translated from the Danish by KATHARINE A. BURKE, B.Sc. (Lond.), Assistant in the Department of Chemistry, University College, London. 39 Paternoster Row. London; New York; Bombay and Calcutta: Longmans, Green and Co. 1908. pp. 495. Price, $2.50.

It is fortunate that these classical investigations of Thomsen have been made a part of the Ramsay series of Text-books of Physical Chemistry, and this leads us to raise the question, What is the object of thermochemical measurements?

It is well known that we have no means of measuring directly the amount of intrinsic energy contained in elements or compounds. Since all chemical reactions are caused by a difference in the potential or the quantity of intrinsic energy present in the reacting substances, it is of the utmost importance for the science of chemistry that we should have some method of measuring at least the difference between the intrinsic energy of the substances before they react, and of the compounds formed.

When two substances are brought together which can react chemically, a part of their intrinsic energy is transformed, in a positive or negative sense, into heat. There is always a thermal change whenever any chemical reaction takes place. The amount of heat set free in a positive or negative sense is a measure of the energy change which takes place and which is the cause of the rearrangement of the system or systems into new systems which are stable for the new conditions; it is the cause of all chemical reactions.

We measure the heat of reactions, then, in order to learn the magnitude of the energy change. This assumes, of course, that there is no electrical energy and no light energy produced during the reaction and that no appreciable work done as is usually true.

Thermochemical measurements center around two laboratories, that of Berthelot, in Paris, and that of Julius Thomsen, in Copenhagen. The results obtained by Thomsen are, taken as a whole, certainly the most reliable that we have in this field, although it was Berthelot who reached the most important generalizations in this field.

We are fortunate in having thermochemical results brought together by one of the two great masters in this field, and brought together in such a convenient and accessible form. The larger divisions of the book are: Formation and Properties of Aqueous Solutions; Compounds of Nonmetals; Compounds of Metals with Nonmetals and Organic Substances.

The book is written in a very readable style, and we can, therefore, assume that the translation has been well done.

H. C. J.

DAS LACHGAS. Eine chemisch-kulturhistorische Studie. Von DR. ERNST COHEN. Mit 31 Autotypen im Text und einer farbigen Karikatur. Leipzig: Engelmann. 1907. pp. 99. Preis, M. 3.60.

A few years ago one of Gillray's caricatures, which is reproduced in the frontispiece of this pamphlet, came into the hands of the author. He has since spent a good deal of time in trying to determine its significance. As a result

of his researches he has learned much that is interesting regarding the "histoire intime" of laughing gas and now presents his results to the chemical public. The caricature is amusing though vulgar. To quote from a description published in 1802: "Most of the figures are portraits of the members of the (Royal) Institution. The gentleman experimented upon is Sir J. C. Hippesley; the operator, Dr. Garnet. The bellows are held by Sir Humphrey Davy, not then a baronet. To the extreme right (to the left of Davy) Count Rumford is easily recognized." Careful inquiry has revealed the fact that "the operator" depicted is not Dr. Garnet, but Dr. Thomas Young. The picture is intended as a burlesque on the Royal Institution.

It is interesting to note that the speculations of Samuel Latham Mitchill, professor of Natural History, Chemistry, and Agriculture in Columbia College, New York, had something to do with directing the attention of Davy to the study of the dephlogisticated nitrous gas (laughing gas) of Priestley. In 1796, Mitchill wrote to Rev. Dr. Henry Mehlenberg (Mühlenberg) in Lancaster, Pa., a letter "On septon (azote) and its compounds, as they operate on plants as food and on animals as poison." According to Mitchill's septon theory "dephlogisticated nitrous gas" or "oxide of septon" (nitrous oxide) is the cause of all infectious diseases. This letter came into Davy's hands and set him to work on nitrous oxide.

Mitchill was a very remarkable man. The universality of his knowledge is thus described: "He was now engaged with the anatomy of the egg, and now deciphering a Babylonian brick; now involved in the nature of meteoric stones; now in the different species of brassica; now in the vaporization of fresh water; now in that of salt; now scrutinizing the geology of Niagara; now anatomizing the tortoise; now offering suggestions to Garnet, of New Jersey, the correspondent of Mark-Akeuside, on the angle of the windmill; and now concurring with Michaux on the beauty of the black walnut as ornamental for parlor furniture; now with his cinchological friend, Samuel Akerly, in the investigation of bivalves; and now with the learned Jewish rabbi, Gershom Seixas, in exigetical disquisitions on Kennicott's Hebrew Bible. Now he might be waited upon by the indigent philosopher, Christopher Colles, to countenance his measures for the introduction of Bronx river into the city; and now a committee of soap boilers might seek after him to defend the innoxious influence of their vocation in a crowded population. For his services in this cause of the chandlers, Chancellor Livingston assured

him, doubtless facetiously, by letter, that he deserved a monument of hard soap.... In the morning he might be found composing songs for the nursery; at noon dietetically experimenting and writing on fishes, or unfolding to admiration a new theory on terrene formations; and at evening addressing his fair readers on the healthful influence of the alkalies and the depurative virtues of white washing."

Dr. Cohen's pamphlet is very interesting reading and from the historical point of view there is much that is valuable contained in it. I. R.